THE ROLE OF THE VASCULAR RESPONSE TO MICROMOVEMENT IN THE HEALING OF EXPERIMENTAL FRACTURES

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ABSTRACT

The two most important elements in the clinical management of long bone fractures are generally considered to be the state of the vascular supply to the bone fragments and the requirement for mechanical stability. The contribution of each of these factors has been extensively studied independently, but their relative significance in the early phase of the healing process remains poorly defined. Recent evidence that intermittent micromovement at the fracture site may enhance the rate of union under experimental conditions has yet to be effectively demonstrated in clinical practice, particularly in high velocity injuries where bony comminution and soft tissue damage may be prominent.

The objective of this thesis was to quantify the role of the vascular response to different mechanical environments, with and without suppression of musculoperiosteal collateral blood flow, after a transverse two-millimetre osteotomy of the ovine tibia which was held in an instrumented external fixation device. Three groups of skeletally mature three year old female Scottish blackface sheep were used for the study. In the well-vascularised groups the axial fixation stiffness was 460 N/mm and 240 N/mm respectively; in the devascularised group it was 240 N/mm and after subperiosteal exposure, a silicone rubber sleeve was interposed circumferentially between cortex and muscle. Outcome was assessed by in vivo measurement of fixator axial load and ground reaction force, calculation of axial interfragmentary gap strain and external counting of dynamic uptake of Technetium-99m methylene diphosphonate at the osteotomy site. Regional blood flow was determined using the radioactive tracer microsphere technique at 14 and 42 days after osteotomy. Post mortem, the isolated tibiae were mechanically tested in torsion and cross sections taken adjacent to the interfragmentary zone, from which area, perimeter, porosity and mineral apposition rate in cortex and callus were calculated.

In the well-vascularised groups the greater rigidity of fixation facilitated significantly greater ground reaction forces at fourteen days after osteotomy, with the result that calculated axial micromovement was 0.8 mm (40% strain) compared with 1.2 mm (65% strain) in the less rigid group. This modest difference in gap strain was associated with a fourfold higher cortical and medullary blood flow at 14 days ($p<0.005$), and greater periosteal cross-sectional area and intracortical porosity at 42 days ($p<0.05$). Ultimately, the haemodynamic changes and distribution of new bone did not correlate with mineral uptake or appositional rate, or with torsional properties, which were not significantly different. Devascularisation, however, resulted in persistently high fixator loads and osteotomy gap strain, and delayed recovery of normal ground reaction force. Cortical blood flow remained at basal levels despite elevated medullary blood flow, which was associated with endosteal resorption and reconstruction by a core of medullary callus, but this contributed little to recovery of torsional properties by 42 days.

It was found that in a well-vascularised fracture model, doubling axial fixation stiffness produced only a 25% difference in initial displacement, which nonetheless had a marked effect on local haemodynamic responses. The vascular changes appeared to precede and determine the osteogenic process, by which a similar structural quality was achieved by different histological pathways. Suppression of the musculoperiosteal reserve resulted in extensive cortical devascularisation and delayed healing in terms of all parameters assessed. It is concluded that the early phase of healing is important in determining the rate of union, but that the recovery of extrinsic collateral blood flow is the limiting factor. A hypothesis is presented which attempts to explain the role of the vascular response to the mechanical environment, and the implications of this study for the clinical management of diaphyseal fractures are discussed.

Statement of Originality

I hereby declare that this thesis has been composed entirely by myself. The experimental work to which it refers was conducted in collaboration with other members of the Department of Orthopaedic Surgery, with myself as the principal investigator. I must specifically acknowledge the contribution of Edward Draper, BSc (Hons), Bioengineer, who designed and manufactured the experimental fixation and torsional testing devices, and created the software for the in vivo biomechanical measurements. Accordingly, the specifications and data on the mechanical performance of these devices are included in his own thesis, in preparation for submission to the University of Strathclyde, and are only referred to in general terms in the present work.

Andrew Lachlan Wallace.

'...there is danger inherent in the mechanical efficiency of our modern methods, danger lest the craftsman forget that union cannot be imposed but may have to be encouraged. For a bone is a plant, with its roots in the soft tissues, and when its vascular connections are damaged, it often requires, not the technique of a cabinet maker, but the patient care and understanding of a gardener.'

Gathorne Robert Girdlestone
The treatment of fractures in the light of their ischaemic complications.
Journal of Bone and Joint Surgery, 1932 14A: 755-762

'Had anyone meditated on balistic machines, and battering rams, as they were used by the ancients, though he might have spent his whole life in the pursuit, yet would he never have hit upon the invention of flaming engines, acting by means of gunpowder: nor would any person, who had made woollen manufactories and cotton the subject of his observation and reflection, have ever discovered thereby the nature of the silkworm or of silk.'

Francis Bacon
Novum Organum, 1620

'If we study Japanese art, we see a man who is undoubtedly wise, philosophic and intelligent, who spends his time doing what? In studying the distance between the earth and the moon? No. In studying Bismarck's policy? No. He studies a single blade of grass. But this blade of grass leads him to draw every plant and then the seasons, the wide aspects of the countryside, then animals, then the human figure. So he passes his life, and life is too short to do the whole.'

Vincent van Gogh
Letter to Theo van Gogh, Arles, September 1888

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The substantial experimental work conducted by Professor Hughes and Dr Ian McCarthy was the fundamental stimulus for the present study. In developing the idea from these origins, and making it a reality, I also owe a great deal to Robin Strachan, now Senior Lecturer in Orthopaedic Surgery in the Royal Postgraduate Medical School, whose enthusiasm, encouragement and advice was always appreciated and extremely valuable. On the practical side, I am indebted to Bobby Fleming and Betty Wyatt for their technical advice, expertise and instruction in the conduct of the experimental investigations. Dr Hamish Law, Honorary Senior Lecturer, took over as my Supervisor in the University of Edinburgh during the final preparation of the thesis, and I am grateful for his guidance, and the willing assistance of the Departmental Secretary, Mrs Cathy Darlington.

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Finally, and most importantly, I would like to dedicate this thesis to my wife, Helen, and my two beautiful daughters, Rennie and Freya, who together provided me with the inspiration to finish, and who continue to make it all worthwhile.

A.L.W.

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1. INTRODUCTION

The restoration and maintenance of skeletal integrity is of profound importance for the normal function of an individual, in terms of both the primary mechanical role of the skeleton in the provision of structural support and locomotion, and the secondary metabolic role of mineral homeostasis.

The repair of a fracture, which may be defined as a loss of continuity of bone as a consequence of traumatic injury, is a remarkable process in that regeneration appears to be morphologically related to developmental events in the embryo (Ogden 1980). In contrast to other connective tissues such as cartilage, muscle or skin which frequently heal with formation of scar tissue, under ideal conditions bone has the potential to result in perfect reconstitution.

Understanding of the mechanisms occurring at both the organ and cellular levels in bone is therefore of great value in the treatment of fractures in man and in animals. However this knowledge has proved elusive and despite technological advances in the design of implants for fracture surgery, controversy still exists over the relative importance of mechanical and biological factors in the healing of fractures and prevention of complications. The introduction to this thesis discusses the most significant problems in the clinical management of fractures, and how this has prompted the experimental investigation of mechanical and biological factors affecting bone healing.

1.1 Clinical management of fractures

1.1.1 Epidemiology

Fractures, particularly of long bones such as the femur, tibia and humerus, represent a major proportion of acute hospital admissions, especially in younger patients, due to sporting, industrial and road traffic accidents (Sahlin 1990a, Bradbury 1990). It has been estimated that in the United States, over 50 million musculoskeletal injuries requiring acute care occur annually, of which more than 10% are due to fractures (Holbrook et al 1984).

In Scotland in 1988, hospital admissions for injuries and poisonings (86 386 admissions) exceeded those for heart disease (63 416 admissions), or cancer (60 608 admissions) or respiratory disease (60 822 admissions) in both males and females of all ages (Scottish Health Statistics 1989). Of this group of injuries and poisonings, more than one-third of admissions were due to fractures, representing nearly 5% of total hospital admissions. In the working population (15-64 years), fractures, particularly in males, accounted for one of the highest rates of admission, comparable with those for either respiratory or gastrointestinal diseases. When examined across all age groups, the distribution of fracture admission rates showed a bimodal pattern with peaks in childhood/adolescence and old age (Figure 1.1).

Holbrook et al (1984) quantified the estimated annual cost of fractures in the United States, and included direct costs of medical care as well as indirect costs such as administration and loss-of-earnings. In total this amounted to over US$ 18 billion per year, and they found that this was more than six times the estimated cost of lumbar disc disorders, three times the cost of osteoporosis and over twice the cost of arthritis.

Figure 1.1: Distribution of fracture admission rates with age (Scotland 1988)

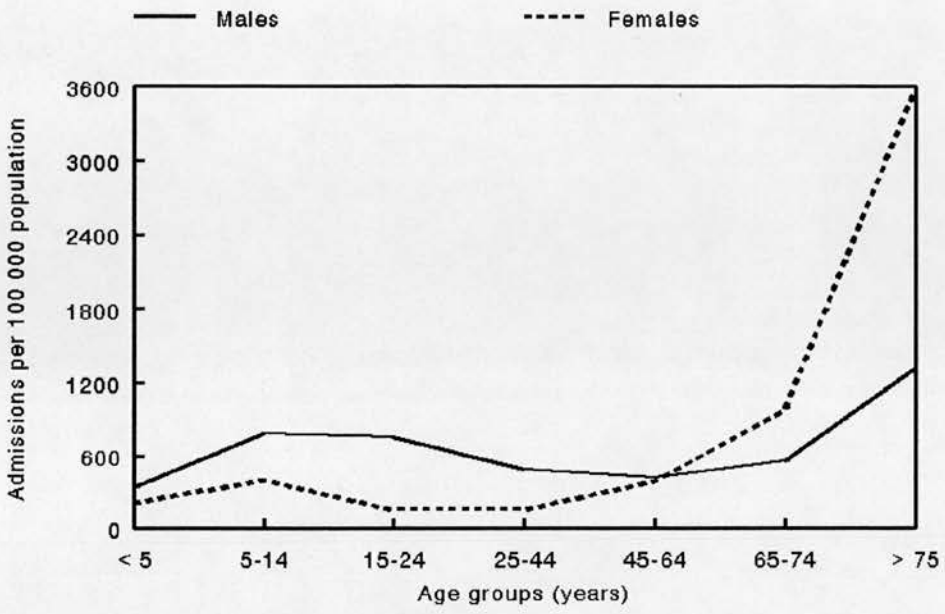


Table 1.1: Classification of tibial fractures (Ellis 1958)

Severity	Type of fracture
Minor	Fragments undisplaced or angulated, with or without minor degrees of comminution or compounding
Moderate	Fragments completely displaced, with or without minor degrees of comminution or compounding
Major	Fragments completely displaced, with major comminution or major compounding

1.1.2 Classification of tibial fractures

Fractures of the tibia and fibula are common long bone injuries (Holbrook et al 1984, Sahlin 1990b) and for many years have served as the clinical model for debate concerning the most appropriate method of fracture treatment. Generally, comparison of clinical studies is difficult because of differences in the site, configuration and displacement of the fracture, and the associated soft tissue damage. The tibia is particularly predisposed to injury because of its prominent subcutaneous border and relative lack of soft tissue coverage compared to the femur, which is most apparent in the distal third of the bone.

Fractures have traditionally been defined as either closed (simple), where the skin overlying the fracture remains intact, or open (compound), where the possibility of environmental contamination exists due to an associated wound, and there is the potential complication of infection (Apley and Solomon 1984). In a survey of 576 tibial shaft fractures managed predominantly with plaster casts, Ellis (1958) made one of the first attempts to classify fractures based on the initial injury, and recognised that the severity of the injury was related to the speed of healing (Table 1.1).

Ellis' classification did not, however, discriminate between different degrees of displacement or types of open wounds, and in recent years Gustilo and co-workers (1976, 1984) have retrospectively and prospectively reviewed the incidence of open fractures in long bones in two large series. Their classification, which is based upon different clinical prognosis, has gained wide acceptance in the orthopaedic literature (Table 1.2).

While soft tissue damage is obvious in open fractures, the signficance of the extent of soft tissue injury in closed fractures has not been widely appreciated. Oestern and Tscherne (1984) have established a classification for closed fractures which takes into

account the severity of injury to both bone and surrounding soft tissues (Table 1.3) which is related to clinical prognosis (Court-Brown, Christie and McQueen 1990).

1.1.3 Complications

Problems in the management of tibial fractures include loss of reduction, malunion, delayed union or nonunion, local swelling, joint stiffness, refracture, compartment syndrome, sympathetic dystrophy, superficial infection and most significantly, osteomyelitis (Owen and Tsimboukis 1967, Hutchins 1981, Waddell and Reardon 1983, Merchant and Dietz 1989). These complications may either be a consequence of the initial injury or of the choice of fracture treatment.

Apart from difficulties in comparison of different groups of fractures, meaningful interpretation of outcome is hampered by the inaccuracy of defining the state of fracture union. It has been claimed that experienced surgeons can detect angular displacements of the order of two degrees at the fracture site (Matthews, Kaufer and Sonstegard 1974), but the elicitation of tenderness to palpation, or discomfort on stressing the bone, during manual examination is highly subjective. Assessment of radiological changes, a standard investigation, has also been shown to correlate poorly with the biomechanical properties of healing bone (Panjabi et al 1985).

Despite these qualifications, clinical reports have identified several factors associated with delay in union. In his classic review of 705 tibial shaft fractures conservatively managed with plaster casts, Nicoll (1964) found that the most important factors were infection, initial displacement, comminution, and associated soft tissue wounds. He reported a delayed union rate (greater than 20 weeks to union) of 17% and a nonunion rate (failure of union with conservative treatment) of 5%. In his series 25% of patients were

Table 1.2: Classification of open fractures (Gustilo et al 1976, 1984)

Type	Description of fracture wound
I	Clean wound, less than one centimetre long
II	Wound without extensive soft tissue damage, flaps or avulsions, more than one centimetre long
IIIA	Wound with extensive soft tissue laceration or flaps, but with adequate soft tissue coverage of bone, or high energy trauma irrespective of the size of the wound
IIIB	Wound with extensive soft tissue injury with periosteal stripping and bony exposure, usually associated with massive contamination
IIIC	Wound associated with arterial injury requiring repair

Table 1.3: Classification of closed fractures (Oestern and Tscherne 1984)

Type	Description of fracture and soft tissue injury
C0	Simple fracture caused by indirect violence, absent or negligible soft tissue injury
CI	Mild to moderate fracture with superficial abrasion or contusion caused by fragment pressure from within
CII	Moderate to severe fracture caused by direct violence, associated with deep contaminated abrasion, and localised skin or muscle contusion; impending compartment syndrome
CIII	Severe or comminuted fracture with severe crushing or contusion of muscle and skin, subcutaneous avulsions, compartment syndrome or vessel rupture

recorded as having significant residual stiffness in one or more joints, and more than 8% had angulation in one or more planes of greater than ten degrees. These findings were supported in a recent long-term followup study by Merchant and Dietz (1989), although in their study clinical outcome appeared to be unaffected by the degree of angulation.

1.1.4 Treatment of closed fractures

The distribution of closed and open tibial fractures varies between centres; in Edinburgh approximately 70% are closed injuries (Hutchins 1981), while in Essen, Germany (Rommens and Schmit-Neuerburg, 1987) the incidence of closed fractures was found to be only 44%, the higher proportion of open fractures being associated with direct trauma, in 80% of cases.

There is a longstanding debate centred on the relative merits of conservative treatment with plaster casts or functional braces versus internal fixation with plates or intramedullary nails for closed fractures.

Sarmiento (1970) has reported consistently good results with patellar tendon-bearing casts, and also with functional braces which allow partial mobilisation of the knee and ankle joints during the process of healing. In a recently reported series of 780 closed and open fractures, 25% developed varus angulation of more than five degrees, with 2.5% nonunion and an average healing time of 17.4 weeks for closed fractures and 21.7 weeks for all types of open fractures (Sarmiento et al 1989).

Compression plating has also been advocated for diaphyseal fractures to achieve anatomical reduction and allow early mobilisation of adjacent joints, but there is a significant risk of skin necrosis (9.5%) and osteomyelitis (2.5%) using this technique (Rommens and Schmit-

Neuerburg, 1987) which also requires considerable soft tissue dissection at the fracture site. Plate positioning may be limited by the extent of the soft tissue lesion (Gotzen and Haas 1984). Other problems include subplate osteoporosis and subsequent refracture after plate removal (Perren et al 1988).

Improvements in the design of intramedullary nails and the development of closed techniques of insertion, and the use of interlocking screws, have led to a resurgence of interest in this method of fixation, particularly for displaced fractures (Alho et al 1990). Comparable union times of 16.7 weeks with low rates of infection (1.6%) have been achieved in closed and Grade I open fractures (Court-Brown, Christie and McQueen, 1990) although knee pain occurred in 40% of patients which may necessitate nail removal. In a randomised prospective trial of 62 displaced closed fractures, Hooper, Keddell and Penny (1991) compared conservative treatment with intramedullary nailing, and found significantly lower rates of angular deformity, shortening and time off work in the nailed group, with no increased incidence of infection.

1.1.5 Treatment of open fractures

The optimal treatment of these injuries remains a challenging problem, especially in Grade IIIB and IIIC fractures in which infection may be present in 50% of cases and the risk of amputation can be as high as 40% (Gustilo, Mendoza and Williams 1984). Grade I open fractures have a prognosis similar to closed fractures, but severe tibial injury has been associated with a rate of delayed union of 50% (Hutchins 1981) and failure to unite in over 25% of cases (Rosenthal, McPhail and Ortiz 1977). The reported incidence of infection associated with primary internal fixation of open fractures varies from less than 5% (Rommens and Schmit-Neuerburg 1987) to more than 19% (Gustilo and Anderson 1976), with an average of 7.5%, calculated from a review of five published series totalling 505

fractures (Worlock 1989).

External fixation has been found to reduce the incidence of deep bone infection and has gained firm acceptance in the management of open fractures (Karlstrom and Olerud 1977, Edge and Denham 1981, Larsson and van der Linden 1983). By virtue of the arrangement of pins driven into the bone at a distance from the fracture site, connected by one or more external bars, the external fixator allows access to the fracture site for debridement of necrotic tissue, wound inspection and supplementary procedures such as partial or full-thickness skin grafts, free myocutaneous flaps, and vascularised pedicle or bone grafts (Byrd, Cierny and Tebbetts 1981, Gordon and Chiu 1988).

However, open tibial fractures managed with external fixation may be complicated by pin track infection and/or loosening (Chan et al 1984, Behrens and Searls 1986) and have also been associated with a high rate of delayed union (Court-Brown and Hughes 1982, Green 1983, Clifford, Lyons and Webb 1987) and it is not clear from the literature whether this is a consequence of excess rigidity of fixation, the severity of the soft tissue injury, or both.

Unfortunately there are few randomised comparative studies of open fractures, but in recent years several authors have promoted the use of more flexible fixators with simple half-frames (Burny in Uhthoff 1980, Schmidt and Rorabeck 1983) or fixators capable of permitting some degree of axial movement of the bone fragments (De Bastiani et al 1984, Kenwright and Goodship 1989, Marsh and Nepola 1990). Kenwright and colleagues (1991) have demonstrated a significant decrease of six weeks in the time to independent weightbearing, in patients with fractures treated with imposed micromovements of up to one millimetre, although this difference was less apparent in comminuted open fractures. In Grade III fractures with severe soft tissue injury, it has been suggested that outcome is independent of the type of device used for external fixation (Court-Brown et al 1990).

In an attempt to minimise any inhibitory effect of external fixation upon healing Blachut, Meek and O'Brien (1990) have recommended delayed intramedullary nailing following several weeks of external fixation, and after soft tissue healing had occurred. This method necessitates a transition period of 1-2 weeks after removal of the frame and before intramedullary nailing, and is contraindicated if there is evidence of antecedent pin track infection, because of a 25% risk of deep infection (Maurer, Merkow and Gustilo 1989). Loose-fitting, unreamed intramedullary nails have shown promising results in Grade I and II open fractures, with a lower incidence of delayed union (14%) and infection (7%) than external fixation (Holbrook, Swiontkowski and Sanders 1989).

1.2 The process of fracture repair

The continuing discussion of the definitive device for clinical management of tibial fractures, and the prevalence of complications associated with all methods of fixation, demonstrates the considerable confusion over the inter-relationship of mechanical and biological variables in healing bone. It is perhaps appropriate to consider the natural history of fracture healing, which has been extensively studied in animal models, before a more detailed examination of the role of regional haemodynamics and axial strain in the biomechanical environment of the fracture.

1.2.1 Histological stages of healing

Classical descriptions have usually been based on the manual production of fractures in long bones or ribs of small animals such as rats and rabbits, in which the fracture fragments have been unsupported (Ham and Cormack, 1979). In rodents healing occurs rapidly, but Sevitt (1981) has argued that the histological changes seen may not always be

applicable to human bone.

Nonetheless, in general three stages of fracture healing have been recognised (McKibbin, 1978). After the initial injury, an acute inflammatory stage ensues which is characterised by necrosis of cells at the margins of the bone fragments and the presence of a haematoma resulting from microvascular damage. The role of the haematoma has long been questioned, but recent evidence suggests that it may have intrinsic osteogenic potential. Mizuno et al (1990) inferred osteogenicity from production of bone at ectopic sites after delayed transplantation of samples of haematoma. However, in this study differentiating cells may have migrated into the substance of the haematoma from surrounding tissues in the two to four days prior to transplantation.

During the first few days intense cellular proliferation takes place in and around the fracture gap. Using tritiated thymidine, Tonna and Cronkite (1961) labelled dividing cells in femoral fractures in mice and found a periosteal response as early as 16 hours after fracture, with a peak at 32 hours. Periosteal proliferation was not confined to the fracture site but involved distant areas of the same bone. Medullary activity was less prominent but also peaked within 24 hours.

In the next stage osteogenic repair tissue is established, in which osteoblasts, chondrocytes and fibroblasts appear along with other connective tissue cells and capillary buds, typical of granulation tissue (Sevitt, 1981). This new tissue forms in the medullary cavity and in the viable areas of periosteum, adjacent to the fragment ends, and is termed the callus. As it enlarges, woven bone is initially laid down by apposition on the existing cortex and subsequently by formation of new trabeculae, principally in the periosteal 'collars', which advance toward each other in an attempt to bridge the defect (Urist and Johnson 1943). In peripheral regions of the callus, cartilage may be formed which is later converted to bone by the process of endochondral ossification.

This stage lasts for two to three weeks and is associated with a dramatic increase in local carbohydrate metabolism (Leung et al 1989) and demands upon calcium and phosphate homeostasis which are detectable in the serum (Meller et al 1984). If this 'primary callus response' (McKibbin 1980) is successful in reducing interfragmentary motion sufficiently to allow union, the final phase of remodelling begins.

This has been divided into a 'soft' phase where fibrous tissue within the medulla is finally ossified, and a 'hard' phase which is characterised by the coordinated activity of osteoblasts and osteoclasts (McKibbin 1980). Lamellar bone, which is identified by its regular arrangement of collagen fibres (in contrast to the loose network in woven bone) is deposited on woven bone surfaces by osteoblasts in the fracture gap, and redundant callus is progressively removed by osteoclasts. Remodelling of the fragment ends is achieved by osteoclast-led 'cutting cones' which, followed by osteoblasts and an afferent capillary, form new osteons, which are the functional units of mature cortical bone (Frost 1980).

1.2.2 Origin of osteogenic cells

The exact origin of osteoblasts, the cells lining the endosteal and periosteal surfaces of cortical bone, and which produce osteoid (unmineralised bone matrix) is unknown. The rapidity of their proliferation after fracture indicates that they probably arise from specific stem cells resident in bone (Triffitt 1987). Owen (1985) has hypothesised a stromal cell system, which is distinct from the haemopoietic system of 'colony-forming units' (CFUs) which is responsible for the differentiation of the myeloid series of cells and osteoclasts. This stromal system contains progenitors for reticular cells, adipocytes and fibroblastic cells in addition to osteogenic cells. Such stromal osteogenic precursor cells appear to be resident in the marrow (Simmons 1980, Connolly et al 1989, Haynesworth et al 1991, Lane et al 1991) and periosteum (Tenenbaum 1990) but may also occur in other

tissues, where bone may occasionally be formed by osteoinduction (Chalmers, Gray and Rush 1975).

1.2.3 Mechanisms of osteogenesis

The phenomenon of osteoinduction has been extensively studied since the isolation of bone morphogenetic protein (BMP) from demineralised bone matrix in the 1960s (Urist 1980, Reddi 1985). This protein was shown to be capable of inducing mesenchymal cells to differentiate into cartilage and bone, but the extent to which induction controls bone formation in fracture callus is unclear.

More recently, Reddi (1989) has purified a specific BMP called osteogenin from bone matrix which initiates the cascade of endochondral ossification by directing mesenchymal cells down a chondro-osteogenic pathway. Once initiated, the process appears to be promoted and maintained by local growth factors such as transforming growth factor beta (Triffitt 1987, Reddi 1989) and these have also been implicated in fracture repair (Einhorn 1991, Jingushi et al 1990).

In the sequence of endochondral ossification, calcification of cartilaginous matrix is closely followed by angiogenesis and vascular invasion (Ogden 1980). Normal hyaline cartilage has been shown to contain inhibitors of angiogenesis (Moses, Sudhalter and Langer 1990). At the growth plate in the immature animal and in callus, differentiating cells, osteoblasts producing osteoid and new bone trabeculae are all intimately related to the new vessels, as noted by Trueta (1963). He hypothesised that an angiogenic factor was liberated by degenerating chondrocytes, and that the capillary endothelium itself was responsible for laying down osteogenic precursor cells adjacent to the vessel. Brighton and Lorich (1990) have suggested that the pericyte, a cell which is found in close

proximity to capillaries, may be an osteoprogenitor candidate.

While the exact mechanism of bone formation in fractures is yet to be elicited, the fundamental importance of an adequate blood supply is well established and is quantitatively related to appearance of new bone (McInnis, Robb and Kelly 1980). In order to understand the changes occurring after bone injury, it is first necessary to review the normal vasculature of bone.

1.3 Vascular supply of bone

1.3.1 Normal vascular anatomy: qualitative and quantitative studies

Few aspects of bone research have attracted such long standing controversy as the blood supply of bone. The first systematic description of the vascular supply of long bones was by Langer (1876), but further work in this area was stimulated by the recognition of clinical syndromes of bone necrosis. Kistler (1934) described the four main groups of vessels entering long bones; namely, the nutrient artery, metaphyseal vessels, periosteal vessels, and epiphyseal vessels with which he included vascular contributions from the joint capsule. Using injections of particulate charcoal, he investigated the vasculature of the rabbit femur and produced embolic infarction of the metaphysis in immature bones (in which the epiphyseal plate was still open), and noted that the periosteal supply was reinforced at sites of muscle and ligamentous attachment. Division of nutrient vessels together with periosteal stripping was shown to produce extensive necrosis of the femoral shaft in rabbits by Foster, Kelly and Watts (1951), which was more slowly revascularised in mature than in immature animals.

However, the advent of microangiographic techniques using intravascular injections of

radio-opaque material allowed the first systematic descriptions of the blood supply of long bones. In a series of experiments in the rabbit, Brookes and Harrison (1957) demonstrated the tibial nutrient artery arising from the anterior tibial artery, and obliquely entering the medullary cavity via a cortical canal, thereupon branching superiorly and inferiorly to supply both the marrow and cortical diaphysis. The metaphysis appeared to receive contributions from the medulla, epiphysis and periosteum. Further observations after ligation of the nutrient artery of the rabbit tibia (Brookes 1960) led him to propose a general theory of circulation in bone (Brookes et al 1961), in which flow was directed centrifugally from the nutrient-medullary vessels outward to the cortical capillaries, draining eventually into either the periosteal venous network or the central medullary sinus. He ascribed little importance to the periosteal arterioles in normal bone, but suggested that the periosteal circulation formed a collateral route, capable of centripetal flow in the event of medullary obstruction (Brookes 1960).

Existence of a similar arrangement in man was demonstrated by Nelson et al (1960), again using microangiography in the amputated limbs of patients with malignant disease, in which there was little evidence of significant periosteal arterial supply. However, in an elegant study involving selective suppression of the nutrient, metaphyseal and periosteal vessels, Trueta and Cavadias (1964) concluded that the periosteal vessels contributed one-quarter to one-third of the blood supply of the cortical diaphysis of the rabbit radius. In similar experiments, Larson et al (1961) and Silberman, Sola and Cabrini (1967) examined the vascular distribution after periosteal stripping of the canine femur, with and without interposition of a teflon or polyethylene sheet between cortex and periosteum. In the immature animal, diaphyseal necrosis occurred; in the mature animal the absence of the growth plate allowed revascularisation of the diaphysis, indicating a second collateral route of medullary supply from the epiphyseal-metaphyseal vessels.

The structure of the cortical microcirculation has been investigated more recently. Trias

and Fery (1979) found that in the dog, the primary network was oriented in the long axis of the bone, in association with the Haversian canals. A secondary, radial network was also observed from endosteum to periosteum, which anastomosed with the primary network in the middle layers of the cortex. In the outer third of the cortex, the radial vessels communicated with periosteal arterioles.

The predominantly longitudinal arrangement of capillaries seems to be an essential feature of mature Haversian bone, and has even been observed in the fossilised bones of dinosaurs (Pawlicki 1983) with remarkable similarity to modern mammals. Lopez-Curto, Bassingthwaighte and Kelly (1980) showed that the separate cortical and medullary capillary beds lie in parallel, describing the radial vessels as 'conduits' to distinguish them from the 'exchange' vessels of the Haversian canals.

Anatomical descriptions based on injection studies are useful from a qualitative point of view, but are subject to error due to direct effects of the injectate and variable perfusion pressure, and may overestimate the functional state of the microcirculation by opening up capillary beds which may be in a 'resting' state with minimal perfusion in the normal animal. The introduction of the radiotracer microsphere technique has made accurate quantitation of blood flow possible in experimental animals (Lunde and Michelsen 1970, Morris and Kelly 1980, Jones et al 1982). It now appears that cortical blood flow is normally 2-10 ml/min/100g in the diaphysis, 10-40 ml/min/100g in marrow and 20-50 ml/min/100g in cancellous bone (Schnitzer et al 1982). In the immature animal cortical and cancellous flows are significantly higher, reaching a maximum in the metaphysis. Within mature diaphyseal cortex, there is evidence of considerable heterogeneity of blood flow, which may be associated with regional remodelling activity (Whiteside, Simmons and Lesker 1977, Willans and McCarthy 1991).

Haemodynamic studies of intact bone suggest that the skeleton receives about 10% of

cardiac output, and that vessels in bone and marrow are responsive to neurohumoral stimuli, particularly those resulting in vasoconstriction (Hughes and McCarthy 1990). Hypotension, hypoxia, noradrenaline infusion and exercise are known to cause increased vascular resistance in bone (Gross, Heistad and Marcus 1979) although other studies have shown increased bone blood flow after treadmill activity in dogs (Tondevold and Bulow 1984). Adrenergic, cholinergic and prostaglandin receptors have recently been implicated in bone (Brinker et al 1990, Cochrane, Fleming and McCarthy 1990) but the precise control of bone circulation remains uncertain.

1.3.2 Revascularisation of fractures: qualitative studies

As long ago as 1923 Kolodny argued that damage occurring to intra-osseous vessels was not the most important determinant of the rate of fracture healing, rather, it was the extent of damage to the periosteal circulation which led to the high incidence of nonunion in his experiments on the canine radius. This view was supported by Haldeman (1932) who attributed the chief role in fracture healing to the periosteum, but also felt that periosteal interposition between the bone fragments could lead to nonunion.

The significance of vascular anastomoses between cortex, periosteum and muscle was highlighted by Zucman (1960) who showed that devascularised muscle could be revascularised from its bony attachment, particularly if the surrounding periosteum was stimulated by elevation at the time of injury. As with intact bone, microangiographic techniques assisted in identification of the specific sources of revascularisation of fractures. Gothman (1961) placed considerable emphasis on the role of adjacent soft tissues as the origin of the vessels supplying the external callus, and found a maximal response two to three weeks after tibial fractures treated with intramedullary nails in the rabbit and monkey. In fractures held with encircling wires, in which the medullary vessels were not

so disrupted, the periosteal response was less apparent.

A detailed study of the vascularisation of fracture callus was undertaken by Cavadias and Trueta (1965) in the rabbit radius. They observed a 'sunray' pattern of radiating periosteal vessels in conservatively-fixed osteotomies, which were closely aligned with new bone trabeculae. Suppression of the periosteal supply by stripping and interposition of a subperiosteal polyethylene sheath resulted in delayed union by medullary callus. Ligation of the nutrient artery and section of the metaphyseal vessels had no effect on bone union, leading the authors to conclude that endosteal callus was 'not indispensable'.

This view contrasts to some extent with the findings of Rhinelander (1968). From qualitative studies he gave the dominant role to the medullary circulation, but drew a sharp distinction between the vascular consequences of displaced and undisplaced fractures. In undisplaced canine radial fractures, the nutrient and medullary arteries were preserved and supplied both medullary and periosteal callus. In displaced fractures the medullary arteries were often divided and periosteal vessels, augmented by muscular anastomoses, were the chief source in the early phase of healing. However, if held in stable fixation the medullary vessels regenerated by three weeks and assumed the dominant role thereafter.

1.3.3 The role of periosteum and muscle after fracture

The question of whether devascularisation of adjacent soft tissues may actually result in delayed union of bone is therefore of considerable interest. Periosteal vessels have been shown to dilate within thirty minutes after a manual fracture in the rabbit, and it has been suggested that this response is an essential prerequisite for cellular proliferation (Wray 1963).

Muscles have been classified into three groups according to the extent of their vascular anastomoses (Campbell and Pennefather 1919), and although it is well recognised that muscle is very sensitive to ischaemia, with demonstrable histological changes after only 35 minutes of tourniquet occlusion (Solonen and Hjelt 1968), it has the capacity to fully revascularise within fourteen days (Le Gros Clark and Blomfield 1945). Holden (1972) stated that revascularisation and union of bone was dependent on the time taken for restoration of muscle blood supply. Clinical evidence of delayed union in association with compartment syndrome in tibial fractures (Court-Brown and McQueen 1987) has since been supported experimentally in a rabbit tibial osteotomy by artificial elevation of intracompartmental pressures, causing diminished perfusion of muscle (McQueen, Fleming and Draper 1991).

The anatomic significance of the periosteum in cortical revascularisation may be complemented by a structural role (Oni et al 1990). Several authors have commented on the periosteal 'seal' or 'tube' which may maintain links between the fragments of a fracture, determining the distribution of periosteal osteogenesis (Macnab and de Haas 1974, Eyre-Brook 1984). The 'callotasis' technique of bone lengthening by mechanical distraction of the early callus is dependent upon a viable periosteal sleeve, and destruction of the endosteum does not appear to affect the result (Kojimoto et al 1988).

1.3.4 Quantification of vascular changes after fracture

Wray and Lynch (1959) made the first serious attempt to quantify the vascular response to fracture. Using a rapid setting synthetic polymer, casts of the vascular tree of both hindlimbs of the rat were made after a closed manual fracture of the left tibia. Vascular volume was increased after three days, reaching a peak at nine days, and returning toward the values for the control limb by thirty six days after fracture.

More accurate measurements were achieved in the canine tibia by Paradis and Kelly (1975), who showed using washout studies of radiolabelled iodo-antipyrine that blood flow reached a maximum of 0.1 ml/min/ml bone at ten days after osteotomy, and was still elevated above control values three months later. The rise in blood flow was correlated with increased uptake of strontium-85, a calcium analogue, at the fracture site. These findings were corroborated by Hughes et al (1978) who postulated that increased uptake of bone-seeking mineral was due to capillary recruitment and the presence of immature new bone. Subsequent experiments demonstrated a two to fivefold increase of blood flow (Lavender, Khan and Hughes 1979, McCarthy and Hughes 1984) at two weeks after osteotomy.

The significance of the vascular anastomoses between muscle and periosteum was quantified by Whiteside and Lesker (1978a,b) in relation to healing rabbit osteotomies. Division of these vessels by extraperiosteal dissection and crushing of muscle resulted in a fall in muscle blood flow of up to 80% and retarded union in 90% of cases.

Washout studies, however, require dissection of the nutrient vessel for injection and this may affect flow distribution. The use of systemic injections of microspheres avoids this problem, by distributing according to cardiac output (Gross, Marcus and Heistad 1981). In the dog, ligation of the nutrient artery has no significant effect on cortical blood flow (0.2 ml/min/g) or callus blood flow (0.77 ml/min/g) at two weeks after a plated osteotomy (Strachan et al 1990), indicating the primary role of the extrinsic circulation to bone in early healing.

1.3.5 Models of devascularised fractures

Encouraged by the problems associated with severe tibial fractures in clinical practice, several investigators have observed the progress of vascular recovery after various

devascularising insults in animal models. Reaming with hand or powered instruments is frequently used in preparation of the medullary canal prior to insertion of an intramedullary nail, and this procedure usually destroys the medullary vasculature along with a thin rim of endosteal cortex (Kessler et al 1986).

In qualitative studies on intact rabbit tibiae, Danckwardt-Lilliestrom, Lorenzi and Olerud (1970) showed that reaming produced endosteal avascularity of about 20% of the cortical cross-sectional area, although this was reduced to 6% if simultaneous suction was used. They explained the difference by suggesting that medullary debris could block intracortical canals and delay revascularisation, which otherwise occurred in the suction group in the third week. After reaming alone (without insertion of an implant) of the canine femur extensive cortical necrosis may occur, which may take up to six months to regenerate (Rhinelander et al 1979).

However, other quantitative work in the rabbit and dog has shown that diaphyseal blood flow may be compensated in intact bones by the periosteal network immediately after reaming, but is eliminated by concomitant periosteal stripping (Whiteside et al 1978). Kessler et al (1986) argued that although cortical necrosis of 50-70% occurred after reaming, this did not affect the formation of periosteal callus. This view was supported in a recent study by Braten and colleagues (1990), although they found superior bending strength in nailed rabbit tibial osteotomies that had not been reamed.

Other approaches to devascularised fractures have concentrated on the revascularisation of osteotomised segments. After a double osteotomy of the canine tibia, Olerud and Danckwardt-Lilliestrom (1971) removed and replaced the intermediate segment, holding the reduction under a compression plate. They noted that in such a stable configuration, 75-80% of revascularisation occurred from the medulla. Intracortical revascularisation took place extremely slowly, mainly through secondary osteon formation by cutting cones, and

was not complete after 12 weeks.

In a similar model, Richards et al (1987, 1989, 1991) examined the effect of skin or skin and muscle coverage of devascularised tibial cortex, but asserted that the repair process was initiated by the soft tissue envelope rather than from the medulla. They found greater intracortical new bone formation, cortical blood flow and rate of union in the muscle cover group, and postulated that the enhanced local vascularity augmented the osteoinductive properties of the devascularised bone. This contention was supported by recent work on the healing of diaphyseal segments in the feline tibia, in which the rate of nonunion occurring in grafts isolated from the surrounding muscular bed was higher than in those subject to medullary obstruction or periosteal stripping alone (Nather et al 1990).

1.4 Mechanical influences on healing

1.4.1 Morphological patterns of bone union

In the 1960s the development of fixation plates allowing interfragmentary compression by the AO group in Switzerland meant that fractures of the diaphysis could be accurately and rigidly reduced in anatomical position. It was soon realised that healing of bone by this method was associated with a minimum of external periosteal callus, and was referred to as primary bone healing (Rahn et al 1971). In experimental studies based on transverse osteotomies with a high degree of coaptation of the fragments, direct tunnelling of osteoclasts across the osteotomy line was observed. In areas without absolute contact, 'gap healing' was described, where woven bone was initially deposited perpendicular to the osteotomy line before being tunnelled (Olerud and Danckwardt-Lilliestrom 1968).

This pattern was similar to the remodelling sequence which normally occurs in the later stages of 'natural' healing by external callus (Simmons 1980). The remodelling process caused only a slow decay in compression over an eight week period after fixation (Perren and Rahn 1980). Although some experimental studies showed that healing by this method resulted in a more rapid return of strength and stiffness than in fractures treated with plaster casts (Lettin 1969), rigid plating was subsequently shown to result in cortical thinning and endosteal resorption (Akeson et al 1976). In the rabbit tibia, plating caused a reduction of torsional strength of 50% after 12 weeks, though this appeared to be reversible after plate removal (Stromberg 1980).

Healing of fractures by external callus has been referred to as secondary repair to distinguish it from primary bone healing (Sevitt 1981) but in recent years this terminology has been disputed, and the terms osteonal and nonosteonal preferred (Chao et al 1989), although osteonal remodelling ultimately occurs in both pathways of healing.

1.4.2 Effect of fixation rigidity

In pioneering experiments, Yamagishi and Yoshimura (1955) recognised that the most important mechanical factors acting at the fracture site were those produced by surrounding muscles, and those imposed by the method of fixation. In a series of externally-fixed rabbit tibial osteotomies held using springs, rubber bands and external plates in a variety of configurations, they were able to show that radiographically and histologically, the most rapid ossification of callus occurred with intermittent axial compression of about 500g. Static compression tended to produce callus with abundant cartilage; distraction produced a fibrous callus; and shear forces resulted in both fibrous tissue and cartilage, with a high incidence of pseudarthrosis.

The problems of rigid plate fixation, together with advances in biomaterials research provoked several workers to compare fibre composites and different stiffnesses of traditional steels (Hutzchenreuter et al 1969, Bradley et al 1979, Terjesen and Apalset 1988, Claes 1989). In experimental osteotomies, decreasing plate rigidity was associated with greater bending strengths, increased periosteal callus quantity and less cortical porosis, and this led to the view that bending loads were significant factors in determining fracture healing (Bradley et al 1979), which might otherwise be retarded by 'stress protection' (Akeson et al 1976).

These findings, and the resurgence of interest in external fixation in the 1970s, initiated the concept of an 'optimal mechanical environment' for diaphyseal fracture healing. External fixation was found to result in increased cortical porosity and increased cortical blood flow when compared with conventionally plated osteotomies, and the authors concluded that the effect of rigidity was important (Lewallen et al 1984). Wu and colleagues (1984), also at the Mayo Clinic, compared six-pin external fixation with a four-pin configuration, which was 30-50% less stiffer in axial loading, and found greater callus quantity and pin loosening in the less-rigid mode. However, they were unable to detect any difference in new bone formation, mechanical properties or blood flow at four months after osteotomy. In another study from the same laboratory, Williams et al (1987) compared the effect of one versus two sidebars, and found that although the two-bar mode was biomechanically stiffer, and resulted in more evidence of osteonal crossing at the osteotomy site, again there was no difference in blood flow or torsional strength. They concluded that any effect of fixation rigidity was an early phenomenon, occurring within the first two weeks.

A more thorough attempt to define a 'therapeutic window' of fixation stiffness was undertaken by Gilbert, Dahners and Atkinson (1989). Using a range of sidebars of different materials, equivalent to the extremes of stiffness from a plate to a plaster

cast, they also found an increase in callus quantity but no difference in mechanical properties of healing canine osteotomies, and suggested that rigidity was unimportant until fragment movement was so excessive as to result in nonunion. However, they performed patellar tenotomies to minimise the effect of weightbearing, and this makes comparison with other studies more difficult.

Recent introduction of fixators allowing movement in the long axis of the bone (dynamisation) has only served to further confuse the issue of the relative importance of rigidity in fracture healing (Chao et al 1989). Uncontrolled dynamic compression, initiated at 15 days after a canine tibial osteotomy, showed a higher incidence of contact-type healing, but there was no difference in blood flow, mineral uptake, new bone formation, porosity or mechanical properties when compared with osteotomies maintained in a rigid mode (Aro et al 1990). Later dynamisation of oblique osteotomies (Aro and Chao 1990), or the addition of an additional sidebar in transverse osteotomies (Egger et al 1990) at 30 days after surgery yielded similar results. However, recent evidence of superior torsional properties of osteotomies dynamised at only seven days (Egger et al 1991) lends support to Williams' concept that the early stages after fracture may be the most significant.

Varying rigidities have also been studied in intramedullary fixation. Molster and Gjerdet (1984) compared steel and polyacetal rods in the rat model. At 16 weeks, there was more callus in the flexible group, but only 20% in each group had united, and this was probably due to rotational instability. Canine osteotomies held with unreamed nails with bending stiffnesses ranging from 3-12% of an intact tibia have shown no differences in mechanical properties (McLaren et al 1991). The value of late dynamisation, by removal of interlocking screws at 5 weeks after osteotomy and allowing impaction at the fracture site, has also been challenged in intramedullary fixation (Dagrenat et al 1990).

1.4.3 Strain modulation of bone remodelling

Observations on the relationship of fixation rigidity to the distribution and mechanical properties of fracture callus have paralleled attempts to define the manner in which applied loads influence the architecture and metabolic activity of bones. Like all materials bone deforms under load, and the extent of this deformation relative to the original dimensions may be expressed as strain. Development and application of the rosette strain gauge to bone surfaces has provided important data on physiological strains occurring in the bones of man (Lanyon et al 1975) and animals (Rubin and Lanyon 1982).

Wolff's original observation (1892) that bone forms in response to the mechanical demands placed upon it was confirmed experimentally in the radius of the pig, in which the ulna had been excised (Goodship, Lanyon and McFie 1979). The overloaded radius increased in cross-sectional area, mainly by periosteal osteogenesis, to equal the combined area of the contralateral radius and ulna, and it was noted that the elevated peak surface strains decreased over time toward more 'normal' values (0.002-0.003), which appear to be very similar between species (Lanyon and Bourn 1979).

Detailed investigation of this phenomenon in the functionally-isolated avian ulna model has revealed several important parameters. The number of cycles, magnitude, rate and distribution of strain all influence the drive to remodelling (Lanyon 1984). Unloaded or statically-loaded bones result in decreased bone area and prominent endosteal and cortical resorption (Lanyon and Rubin 1984). It was postulated that the objective stimulus for remodelling is the error between the distribution of applied strains and those genetically determined to be appropriate for the particular location in the bone (Lanyon 1987).

This view contrasts to some extent with that of Carter (1987), who predicted from theoretical studies that the entire morphology and internal structure of skeletal organs

was determined by the loading history throughout growth and development. The mechanism by which mechanical signals are transduced by cells resident in bone is not yet clear, but prostaglandins (Brighton et al 1991) and membrane-bound phospholipase (Sennett et al 1991) have been implicated in this process.

1.4.4 Interfragmentary strain in fractures

The concept of interfragmentary strain was most comprehensively proposed by Perren (1979) in an attempt to explain the different responses of healing bone to different mechanical environments. Based upon the assumption that tissues of increasing rigidity can normally tolerate progressively decreasing strains, he argued that the repair of a fracture is an orchestrated sequence of tissue differentiation which reduces fragment motion in preparation for osteogenesis. Excessive strains in the fracture gap were postulated to initiate fragment end resorption, thereby reducing the relative deformation of the repair tissues (Hutzchenreuter et al 1969). This theory predicts that low strains should result in a minimum of granulation tissue or cartilage, and virtually immediate appearance of new bone.

Motion at the fracture site may occur in six degrees of freedom (Aro et al 1990), but Perren's theory does not predict the most osteogenic plane or direction of movement. Two-dimensional finite element modelling of a transverse diaphyseal fracture has suggested that intermittent compressive stresses are the dominant influence in healing, although revascularisation and new bone would appear to form initially in areas of callus subject to shear stresses (Carter, Blenman and Beaupre 1988).

Experimental studies with controlled mechanical environments have concentrated principally on intermittent axial loading, as this maintains alignment during the healing process.

Wolf et al (1981) compared externally-fixed rabbit tibial osteotomies held in 80 N constant compression with those held in 8 N compression with superimposed cyclic loads of 40 N applied by a pneumatic actuator at 1 Hz for 6 hours per day. Biomechanically, the cyclically loaded bones regained torsional strength more rapidly, though there was no difference between the groups by eight weeks.

The effect of applied strain on fracture callus was more convincingly demonstrated by Goodship and Kenwright (1985). In a transverse osteotomy of the ovine tibia, leaving a 3 mm gap, they compared a rigid external fixation configuration with an imposed regime of micromovement not exceeding 1 mm (33% strain) achieved by the use of sliding clamps and a pneumatic actuator. The pattern of axial loading (up to 360 N) was based on that known to provoke an osteogenic response in intact bone; i.e. a rate of 30 000 microstrain per second for 500 cycles per day at 0.5 Hz (17 minutes in total daily). Micromovement resulted in significantly more periosteal callus, greater torsional stiffness and a more rapid increase of bending stiffness during healing, as determined from strain gauges attached to the fixator bar. In a subsequent study, however, applied displacements of 0.5 mm (16% strain) enhanced and 2 mm (66% strain) inhibited healing when compared with rigid controls (Kelly et al 1987).

Several criticisms can be made, however, of the model used in their experiments. The authors did not estimate or quantify the strains occurring in the rigid mode of fixation; while they stated that the clamps were locked 'in order to prevent any interfragmentary movement', in an ambulant animal with a unilateral fixator, absolute rigidity is unlikely in a relatively large 3 mm gap, and low strains must have occurred at the osteotomy site in response to weightbearing. Similarly in the intervals between treatments the osteotomy in the micromovement group would also be subject to low strains. Their finding, then, of more advanced healing in the group with higher imposed strains is at odds with Perren's hypothesis that low strains favour osteogenesis. In a later study (Watkins et al 1988)

using the same osteotomy model, increasing frame stiffness by 30% from 500 N/mm to 700 N/mm and thereby presumably decreasing micromovement, was associated with a delay in healing as assessed by mineral content and indirect measurement of fracture stiffness.

The other difficulty with models using powered devices to develop strain fields is whether the priority is given to the applied load or to displacement. Under an intermittently applied load of constant magnitude, the progressive ingrowth of tissue into the osteotomy gap will tend to reduce the possible displacement of the fragment ends and consequently reduce the applied strain. Alternatively, displacements may be maintained by increasing the applied load as the healing tissue progressively increases in stiffness. In recent experiments, graduated strain fields in the osteotomy gap have been developed using applied bending moments (Cheal et al 1991, Hente et al 1991) and these have demonstrated bone formation earliest in regions of strain below 20%, although finite element analysis revealed the very complex nature of the strain field in three dimensions.

1.5 Other factors affecting healing

While there is considerable evidence for the role of mechanical and vascular variables in determining the rate and form of fracture healing, it must be acknowledged that there are other less well-studied factors acting in concert. Bassett and Herrmann (1961) found that in vitro cultures of embryonic chick tibia formed bone when subjected to high (35%) oxygen tension, but low tensions (5%) resulted in formation of cartilage. High tensions combined with mechanical stretching caused fibrous tissue to develop. Despite this, Brighton and Krebs (1972) postulated that the relative hypoxia demonstrated at the fracture site using a microelectrode assembly was essential for normal cellular differentiation, and was due to an imbalance of cellularity and vascularity in the first weeks after fracture. Chronic hypoxia, achieved by a cardiac right-to-left shunt ($p_aO_2 < 45$ mmHg), is associated with

abundant cartilage and a high incidence of nonunion in canine fibular osteotomies (Heppenstall, Goodwin and Brighton 1976).

Electrical activity in bone has attracted an enormous amount of research for many years, but the role of electric fields in fracture repair remains poorly defined. Streaming potentials, created by movement of ion-containing fluid past bone surfaces, have been shown to occur in vivo and are related to variations in mechanical stress, and possibly, intramedullary hydrostatic pressure (Gross and Williams 1982, Otter, Palmieri and Cochran 1990). Their role is uncertain, but experimental (Law et al 1985) and clinical studies (Colson et al 1988, Sharrard 1990) using induced electrical currents have shown equivocal results in terms of radiographic and clinical assessment of union.

Neurohumoral mechanisms may also mediate bone healing. Denervation of the sciatic nerve results in enhanced biomechanical properties of healing fractures in the rat fibula (Frymoyer and Pope 1977), but other workers have failed to reproduce this finding, and indeed found that removal of periosteal mechanoreceptors from the rat fibula caused nonunion (Aro, Eerola and Aho 1985). Prominent callus and rapid healing of fractures are a well-reported occurrence in patients with severe head injuries, and release of an endocrine factor may be involved (Bidner et al 1990). Einhorn et al (1990) have also provided experimental evidence for a generalised skeletal osteogenic response within five days of a fracture, and postulated that this was due to a circulating growth factor.

1.6 Objectives of the thesis

It is apparent from the experimental and clinical studies which have been reviewed that both the vascular status and the mechanical conditions to which a fracture is subject are both important determinants of the outcome of the repair process. Qualitative and quantitative investigation of vascular recovery in fracture models has frequently been performed using a wide variety of imposed mechanical environments, from no fixation or plaster casts to plates, nails and external fixators; consequently understanding of the effect of fixation on the vascular response, and the effect of the vascular response on fracture healing, is extremely difficult.

On the other hand, the extensive experimental research on manipulation of mechanical factors has yet to be effectively translated into clinical practice, and one major criticism is that the bulk of this work has taken place using surgically-created fracture models which have pristine vascular environments, where the effect of soft tissue damage on revascularisation is not a significant variable. The mode of fixation chosen for a fracture will affect both the mechanical conditions of the fracture site and regional blood flow.

The purpose of this thesis was to determine the inter-relationship and relative importance of extrinsic and intrinsic haemodynamic changes and axial interfragmentary strain in the controlled mechanical environment of a model fracture, using quantitative techniques of analysis. The ultimate aim of the work was to provide a scientific basis for the appropriate clinical management of long bone fractures, with special reference to high energy injury.

2. DEVELOPMENT OF THE OVINE TIBIAL MODEL

2.1 Introduction

The choice of an appropriate model in which to study the healing of fractures is dependent upon several points. In general, there are six levels of organisation of an organism; subcellular, cellular, tissue, organ, organ system, and whole animal (Roach, Shearer and Archer 1989). Much research on the behaviour of bone has been performed using cell cultures, but this is usually restricted to differentiated cells of a defined morphology. The healing of fractures involves a vast array of cell types, from uncommitted stem cells to chondrocytes, fibroblasts and osteoblasts and this coordinated arrangement would be difficult to reproduce in culture.

Although the effects of mechanical strain have been studied in vitro (Brighton et al 1991), adequate study of the vascular response in bone and surrounding soft tissues necessitates the use of a whole animal model. Rats and rabbits have frequently been used in previous work in this area, however the dimensions of the long bones in these animals limit the sophistication of fixation devices which may be employed, and the loading conditions in small animals are less analogous to man than those occurring in larger mammals such as dogs or sheep. Similarly, fractures or osteotomies in small animals tend to heal quickly and consistently, and therefore subtle variations in mechanical or biological variables which may be very relevant to the treatment of fractures in man may be masked.

2.2 Characteristics of the ovine tibia

For this series of experiments the sheep was chosen as a suitable model. Despite the fact

that much of the quantitative work on blood flow and biomechanical properties of bone has been performed in the dog, these animals are now expensive and difficult to obtain for research purposes, and may be of variable size and uncertain pedigree. Advantages of using sheep were that they were similar in size to the dog, relatively inexpensive, easy to handle and train, and were readily obtainable from local herds of the same breed, primarily bred for domestic consumption. The ovine tibia has been used previously in studies of fracture healing (Goodship and Kenwright 1985).

2.2.1 Regional anatomy

In the sheep, the tibia is the major weightbearing bone of the crus, or leg, and lies between the joints of the stifle (knee) and hock (ankle). Although roughly equivalent in length to the canine tibia (May 1964), the most striking difference is the virtual absence of the fibula, which usually persists as two small components; a small proximal nodule fused to the lateral condyle of the tibia, and the lateral malleolus distally which articulates with the tibia and talus. A vestigial fibular shaft may be present as a fine calcified bar lying laterally in the intermuscular septum but this is inconstant.

The tibia is triangular in cross-section proximally and has a prominent crest which is slightly concave laterally and slopes from the tibial tuberosity distally to meet the shaft, or diaphysis. The diaphysis becomes narrower and elliptical toward the hock, with its long axis lying mediolaterally. Throughout its length, the anteromedial surface of the tibia lies subcutaneously.

The muscular arrangement of the ovine crus is similar to the human leg (Figure 2.1), although the lateral compartment is less well developed because of limitation of movement to the sagittal plane at the tibiotarsal and intertarsal articulations (Dyce, Sack and

Wensing 1987). Muscles of the cranial or anterior compartment extend the medial and lateral digits of the hoof and have no significant attachment to the diaphysis. In contrast, the deep digital flexor, in common with the soleus in man, has an extensive origin on the flattened caudal (posterior) surface of the proximal two-thirds of the tibia.

2.2.2 Vascular supply

The popliteal artery enters the crus behind the stifle joint and divides into the cranial (anterior) and caudal (posterior) tibial arteries. The anterior tibial artery is the principal supply of the distal extremity and passes from the posterolateral border of the proximal tibia diagonally across and in close relation to the diaphysis to lie anteriorly, with the extensor tendons, passing under the extensor retinaculum into the foot (Habel 1970). The posterior tibial artery supplies only the muscles of the flexor compartment.

The blood supply of the ovine tibia has not been well described, and therefore a cadaveric injection of radio-opaque medium was performed to define the major vessels more precisely. From two immature sheep the right hindlimb was obtained post-mortem and the skin and subcutaneous tissues removed. The femoral artery was located on the medial aspect of the thigh and a large bore (16 gauge) intravenous cannula inserted into the lumen and secured. The vascular tree of the limb was then perfused with approximately 100 ml 0.9% saline solution until residual blood had been removed and clear fluid was draining freely from the femoral vein. This was followed by injection of 25 ml 1% toluidine blue dye, which revealed any leaking vessels on the surface of the limb which were then ligated.

A mixture of 15% barium sulphate in 25% gelatine was warmed to 37 degrees, transferred to a 50 ml syringe and injected under firm manual pressure into the femoral artery via the

Figure 2.1: Regional anatomy of the ovine hindlimb

AC, PC = anterior, posterior muscle compartments. T = tibia.

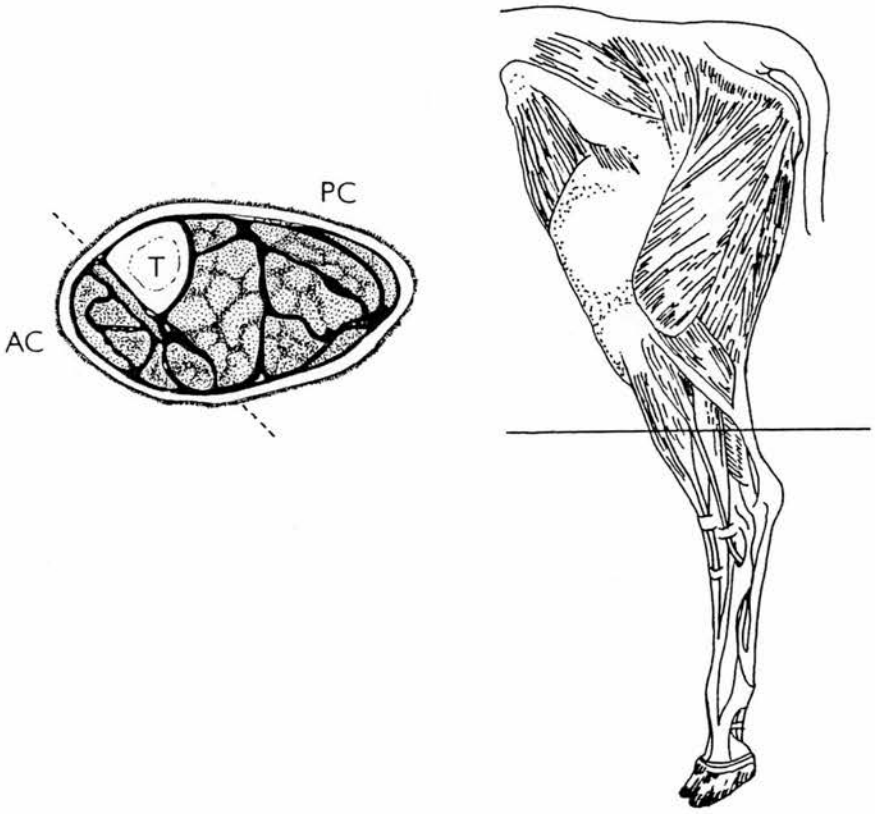


Figure 2.2: Cadaveric angiography

The tibial nutrient artery is indicated by an arrow.

cannula. The injection continued until it was physically impossible to introduce any more contrast medium into the limb. Both limbs were then radiographed in a standard manner in the lateral and oblique planes.

The resulting angiograms were similar in both cases, and the vasculature was best observed in the oblique views (Figure 2.2). The popliteal artery was clearly visible, giving rise to the anterior tibial artery. Prominent vessels to the muscular compartments were seen, branching at right angles. From the anterior tibial artery, a slender nutrient artery, 1.5 cm in length was noted to enter the tibia through a foramen in the posterolateral cortex, with an intracortical course of similar length. Details of the intraosseous circulation were not well revealed by this technique, but it was reasonable to assume that this would be of equivalent morphology to descriptions of the lapine and canine tibial networks.

2.2.3 Morphometry

The osteology of the ovine tibia has been well documented (May 1964) but there is little information in the literature on the typical regional dimensions of the tibia. These data were required for the planning of the osteotomy site and the design and manufacture of the fixation system, and was also obtained from cadaveric measurements.

Six pairs of ovine tibiae were stripped of soft tissue attachments and examined and measured using a standard steel ruler and a Vernier caliper. The results of this analysis are presented in Table 2.1. The tibial length was measured between the articular surface of the femoro-tibial (stifle) joint and the tip of the medial malleolus; the diaphysis was measured between the flares of the metaphyseal regions at each end.

Table 2.1: Cadaveric tibial morphometry

Dimension	Mean in millimetres (+/- SD)
Overall length	188 (+/-7)
Diaphyseal length	140 (+/-5)
Position of nutrient foramen	61 (+/-4)
Width in anteroposterior plane:	
Proximal	25 (+/-3)
Middle	16 (+/-2)
Distal	17 (+/-2)
Width in lateral plane:	
Proximal	24 (+/-2)
Middle	19 (+/-2)
Distal	21 (+/-3)

The diaphysis forms approximately 75% of the tibia, and is most narrow at the junction of its middle and distal third. The nutrient artery enters at the junction of the proximal and middle thirds, and this location appears to be relatively consistent. Allowing for its intracortical course, direct damage to the nutrient vessels would be avoided by an osteotomy in the middle or distal thirds.

2.2.4 Histology

A major concern in the use of animal skeletons to model human fractures is the relationship of the type and structure of bone between species. Currey (1984) has characterised mammalian bone into four types. Woven bone is found in embryonic and neonatal bones and fracture callus, but is transient and ultimately replaced by lamellar bone, which as its name suggests, is composed of orderly layers. He then describes a specialised form of lamellar bone, which makes up the Haversian systems, or secondary osteons. These take the place of primary osteons (originally formed by appositional growth around blood vessels) as a consequence of remodelling later in the life of the animal, but this is a slow process. He argues that in large mammals such as cattle, bone growth must be rapid, and for this purpose a fourth type, plexiform or fibrolamellar bone exists. This is composed of alternate layers of parallel-fibred bone, intermediate in structure between woven and lamellar bone, and true lamellar bone. Depending on the species, this fibrolamellar bone may be remodelled by Haversian systems or remain as the definitive structure; in humans, primates and carnivores it is remodelled, while in cattle and deer the primary laminar arrangement tends to persist, with only small regions becoming Haversian.

The histological appearance of tibial cortical bone was assessed in a mature female sheep of approximately three years of age which had been used for an unrelated experiment. Ten

serial sections, averaging 100 um in thickness, were cut in the transverse plane from the undecalcified diaphysis on a Buehler Isomet low-speed microtome (Buehler Instruments, Lake Bluff, Illinois USA). Each section was fixed for 24 hours in 70% alcohol and stained with toluidine blue, and examined under the light microscope at low power, with and without polarised light.

In the proximal diaphysis he appearances were of mixed Haversian and fibrolamellar bone (Figure 2.3). Towards the middle of the diaphysis, fibrolamellar bone predominated, while in the distal diaphysis, a central zone of Haversian bone was interposed between endosteal and periosteal layers of fibrolamellar bone.

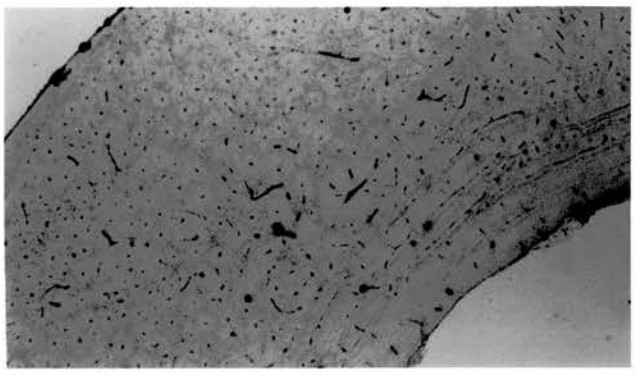
2.3 Method of osteotomy

Previous investigators have used a number of methods to induce a fracture of a long bone. In attempting to reproduce as closely as possible the damage to bone and soft tissue sustained in human fractures, devices which cause a fracture by accelerating an external load against the intact or exposed tissues of the limb have been used (Ashhurst 1986, Einhorn et al 1990), but despite the authors' claims of reproducibility, these may result in a variety of fragment configurations and degree of comminution, with considerable consequences for any analysis of the mechanical factors acting at the fracture site. Similarly, muscle and vascular damage may be highly variable.

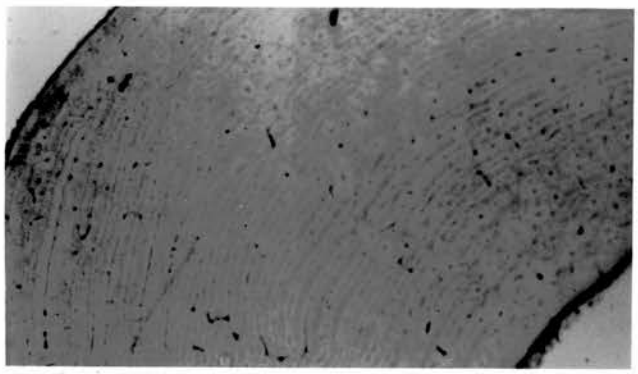
An alternative is the creation of a transverse surgical osteotomy, which is not a fracture, but which allows a more precisely controlled insult to the bone. This may be achieved by oscillating power saws (Cheal et al 1991), or manually with a Gigli saw (Goodship and Kenwright 1985), which consists of twisted strands of fine gauge wire with a handle at either end. For this experiment, the Gigli saw was selected as the best means

Figure 2.3: Histological appearances of the normal ovine tibia

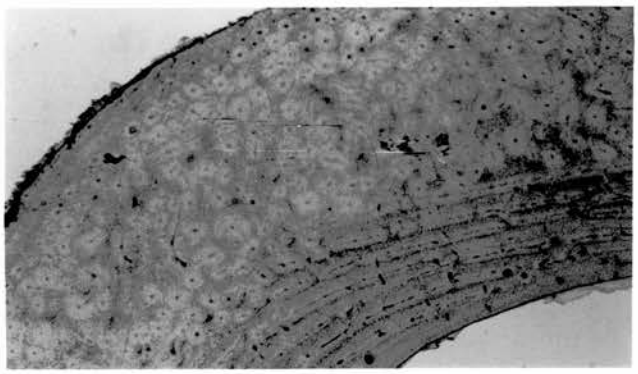
Proximal



Middle



Distal



of ensuring a reproducible geometry whilst preventing adjacent soft tissue damage and the dispersion of saw debris in the vicinity of the osteotomy. A heavy gauge was chosen (Charles Thackray Ltd, Leeds, England) which reliably produced a 2 mm defect in the diaphysis, as determined from trials in cadaveric bone which were measured with a feeler gauge.

2.4 Summary

The ovine tibia and surrounding tissues were found to be highly analagous to the anatomy of the tibia of a young adult human, in terms of anatomical relationships and gross vascular supply, although histologically, there appeared to be proportionately less Haversian bone in the central diaphysis. In relation to the creation of an osteotomy with a Gigli saw, access to the tibia through the anteromedial subcutaneous tissues was possible without significant dissection of muscle or damage to nerves and vessels traversing the limb.

3. EXTERNAL FIXATION AND BIOMECHANICAL ANALYSIS

3.1 Introduction

The search for a dependable, objective, non-invasive method for the assessment of fracture healing has been extensive. Visual interpretation of standard radiographs has been shown to be unreliable (Panjabi et al 1985), and therefore many other techniques have been proposed and advocated, with varying success. These include osteomedullography (Puranen and Kaski 1974), 'shift comparison' radiography (Hammer, Edholm and Lindholm 1984), radioisotope imaging (Smith et al 1987), impedence osteography (Ritchie and Kulkarni 1990), resonant frequency measurement (Benirschke et al 1991), and biochemical tests (Kallio et al 1990).

However, the principal aim of the healing process is the restoration of the structural properties of the bone involved, and because assessment of biomechanical competence by direct measurement of load or displacement is practically difficult in fractures, much interest has centred on the use of implants transduced to measure applied loads (Sell 1989). These have been applied to plates (Perren and Rahn 1980) and intramedullary nails (Michel et al 1991) but their greatest application to date has been in external fixation, which offers the advantage of avoiding direct implantation of metal at the fracture site which might otherwise interfere with the biological response.

3.1.1 Instrumented external fixation

This work was pioneered by Burny and colleagues (Bourgois and Burny 1972) and has been developed experimentally (Kaplan et al 1985, Williams et al 1987) and applied clinically (Harris et al 1984). A fracture with a gap held with a unilateral bar and pins may be

regarded as a system of two loadbearing members in parallel (Figure 3.1). Loads applied due to weightbearing and muscle contraction will initially be carried by the fixator; as the fracture heals, the load on the fixator will diminish. The load on the fixator may be estimated by measuring either the deformation of the bar (Goodship and Kenwright 1985) or the relative movement of the bone pins (Kaplan et al 1985, Cunningham, Evans and Kenwright 1989).

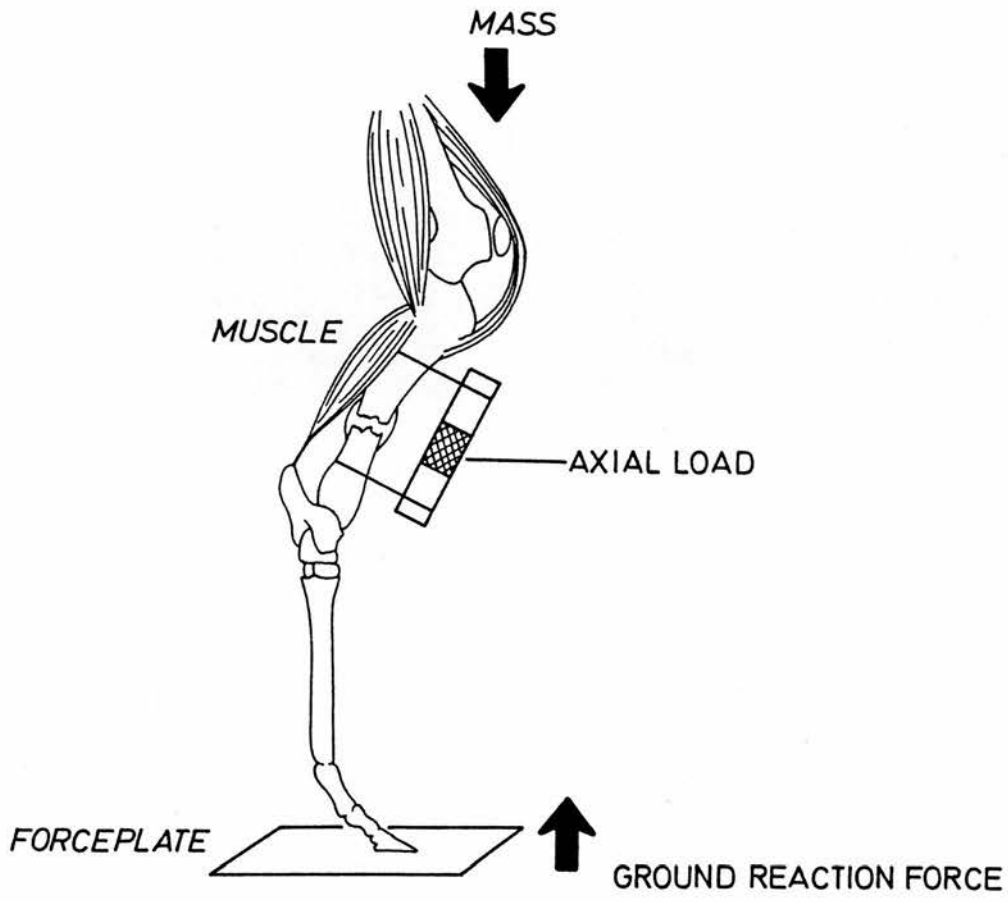
Theoretical and clinical studies have shown that the relationship between callus stiffness and deflection of the fixator bar may be described by a hyperbolic function, alterations in the normal pattern of healing resulting in characteristic curves (Nishimura 1984). Deflections reached a plateau when the callus had reached 50% of the mechanical properties of an intact bone, and this probably defines the limits of usefulness of this method.

Most work in this area has focussed on load (or consequent displacement) occurring in one degree of freedom, most commonly the axis of the fixator, either in tension (Kaplan et al 1985) or compression (Cunningham, Evans and Kenwright 1989). However, considerable displacements in bending and shear at the fracture site may also occur with external fixators in clinical use (Seligson et al 1981) and the effect of micromovement in these planes is not well defined. Pin loosening in vivo may also affect the validity of measurements obtained from instrumented external fixators (Kay, Ross and Powell 1989).

3.1.2 Mechanics of external fixation

In vitro studies have shown that the rigidity of an external fixation device may be increased by increasing the number and diameter of bone pins, the separation between pins within each group, by angulating the pins, and by decreasing the offset distance of the sidebar from the bone (Briggs and Chao 1982). Increasing the number and stiffness of the

Figure 3.1: Measurement of in vivo loads



sidebars will also augment rigidity in all planes, but since stiffness in bending is proportional to the fourth power of the pin radius (or the square of the cross-sectional area), increasing the pin diameter is probably the most critical factor in a unilateral frame (Kempson and Campbell 1981).

In biomechanical analyses of unilateral configurations subject to loads, the least stiffness is generally found in bending in the plane of the pins due to pin flexibility or frame weakness (Chao 1987). Many of these studies have assumed an efficient connection at the bone-pin interface, where cortical bone is cyclically loaded during weightbearing and may even undergo plastic deformation of more than 10% at high loads (Klip and Bosma 1978). Excessive pin-bone interface stress may result in loosening of a single pin with a loss of axial stiffness of 20%, and by increasing the load on the remaining pins, rapidly leading to loosening of a second pin (Churches, Tanner and Harris 1985). Histologically this is characterised by bone resorption and connective tissue proliferation around the pin (Schatzker, Horne and Sumner-Smith 1975).

3.2 Preliminary studies

It was apparent from the literature that an external fixation device would best facilitate prescription of movement and measurement of axial load in vivo, but several design considerations were taken into account in order to minimise error. The definitive experimental device required high stiffnesses in all degrees of freedom other than the axial direction, together with a prescribed axial stiffness and transducer capable of measuring axial loads in the fixator, thereby allowing calculation of axial strain occurring at the osteotomy site. The development, specification and mechanical performance of the fixation device and analysis system is presented in detail elsewhere (Draper 1992).

There are no published quantitative data on axial loads occurring in the osteotomised ovine tibia, and therefore a pilot experiment was carried out to establish the design specifications for the definitive fixator, to assess the feasibility and validity of in vivo monitoring, to define the technique of devascularisation for the haemodynamic studies, and to gain experience with intra- and post-operative management of the ovine model.

3.2.1 Pilot fixation device

A single piece unilateral fixator was constructed which contained strain gauges mounted in the central section, arranged so as to measure deformations of the bar in response to (i) loads in the fixator axis and (ii) bending moments in the plane of the pins (Figure 3.2). The axial load was required to allow the axial stiffness of the definitive device to be defined and to monitor the progression of healing; bending was chosen because it would be the loading mode in which the definitive unilateral fixator would be most likely to fail.

From theoretical analysis, the fixator was designed so that loads of up to 1000 N in other planes of motion would result in combined deflections of approximately less than 1 mm. This high degree of stiffness was achieved with the use of six 110 mm long standard self-tapping pins with tapered threads (Orthofix srl, Verona, Italy), which were sheathed with stainless steel to increase the shank diameter from 6 to 10 mm (Figure 3.3).

3.2.2 Pin torque study

In an attempt to improve the validity of measurements from the instrumented pilot fixator, it was decided that the pins would be regularly tightened to predetermined torque values

Figure 3.2: Pilot fixation device

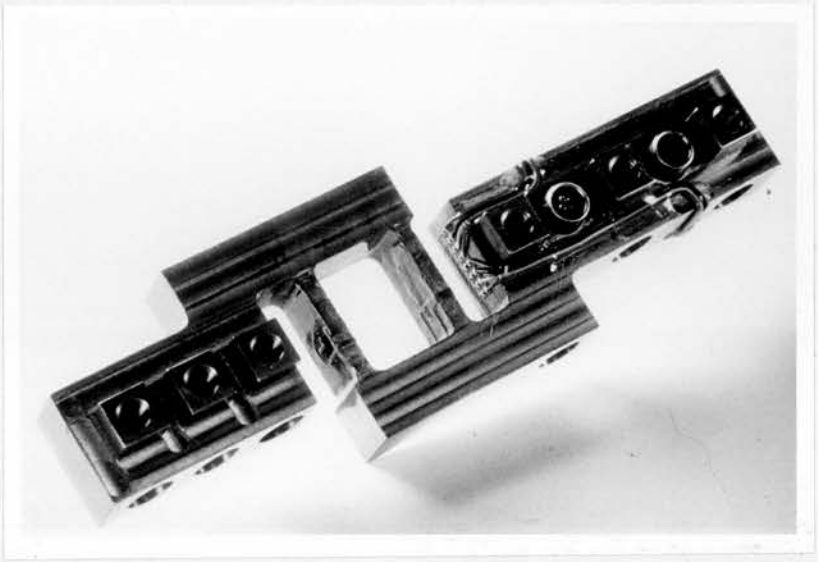
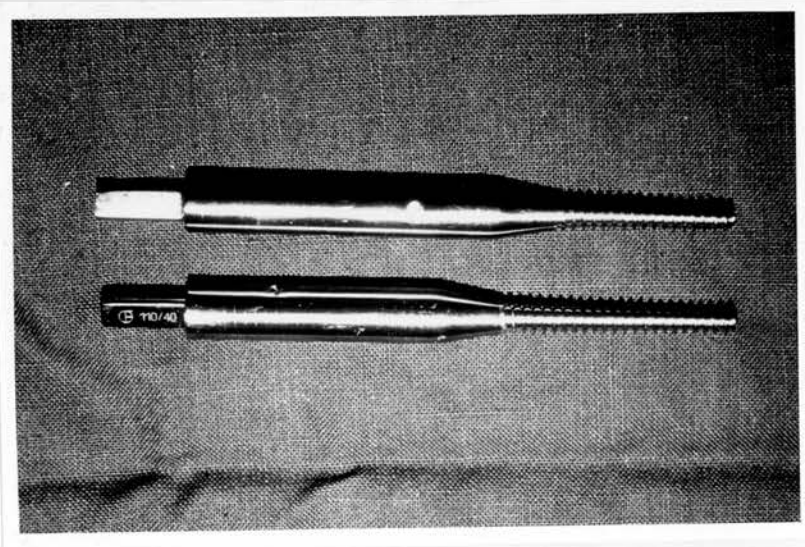


Figure 3.3: Modified bone pins



in the postoperative period. While there are some data in the literature on torques achieved with screws for compression plates (Cordey, Rahn and Perren 1980) this cannot be applied directly to unilateral external fixation, and so an experiment was undertaken in five cadaveric tibiae from adult sheep.

A calibrated torque wrench was used (Torqueleader, MHH Engineering Co Ltd, Bramley, Surrey, England). Pins were driven by hand into predrilled holes in six regions of the tibia from the proximal to the distal metaphyses, and the torques measured when the pin entered the near cortex, the far cortex, and when the pin was felt to be adequately and subjectively 'tight'. The results are presented in Table 3.1. From this study it was evident that the highest torques were achieved in the mid-diaphysis. Thresholds for tightening were set at 2.0 Nm for the two peripheral pins and 2.5 Nm for the four central pins.

3.2.3 Operative procedures

Two mature female Scottish blackface sheep weighing approximately 45 kg were selected and fasted for 12 hours prior to surgery. General anaesthesia was induced with 4% halothane, nitrous oxide (4 L/min) and oxygen (4 L/min) after which the animals were intubated with a No. 9 endotracheal tube. Anaesthesia was maintained at approximately 2% halothane, nitrous oxide (2 L/min) and oxygen (2 L/min) during the procedure.

In each sheep, the right hindlimb was shaved, sprayed with antiseptic (2% chlorhexidine in 70% alcohol), draped in a standard fashion and the position of the stifle and hock joints marked with a sterile pen. The pins were inserted through stab incisions over the anteromedial subcutaneous border of the tibia using a special guide after predrilling with a 4.8 mm bit using an irrigated power drill. The pilot fixator was then applied in the

anteroposterior plane with an offset distance of 60 mm from the centre of the bar to the estimated centre of the tibial shaft.

A longitudinal incision was then made between the two sets of three pins and the periosteum of the anteromedial surface of the tibia exposed. The periosteum was incised transversely and a 2 mm osteotomy created with a Gigli saw. Saw debris was removed by irrigation and suction of 0.9% saline solution. In the first sheep the fascia and skin were then closed in layers using an absorbable 2/0 suture, taking care not to damage any muscle attachments to the tibia. In the second sheep, the periosteum was stripped circumferentially with an elevator and excised for 20 mm proximally and distally from the osteotomy site, before closure. Through a second incision on the lateral aspect of the leg, the tibial nutrient artery was identified, ligated with a non-absorbable suture and divided. These procedures were expected to create a devascularised environment for the healing osteotomy in the second sheep.

3.2.4 In vivo measurements

Postoperatively, both animals were walking and weightbearing on the first day. On the second and seventh days, and every week thereafter, in vivo measurements of axial load and bending moment were made as the animal walked across a forceplate in a specially constructed circuit. The forceplate was sensitive only to the vertical component of the ground reaction force, and was mounted at the same level as the floor. Signals from the forceplate were sent to a BBC Model B microcomputer (Acorn Computers Ltd, Cambridge, England). Signals from the transducers on the fixator bar were sent to the computer by a radiotransmitter located in a canvas harness on the animal's back (Figure 3.4). These signals were then received by the microcomputer, sampled at a frequency of 50 Hz and stored on disk for analysis.

Table 3.1: Cadaveric pin torque measurement (Nm)

Pin site	Near cortex	Far cortex	'Tight'
Proximal			
1	-	0.74 (+/-0.34)	2.42 (+/-0.48)
2	0.42 (+/-0.18)	0.92 (+/-0.29)	2.70 (+/-0.55)
3	0.52 (+/-0.19)	2.28 (+/-0.77)	3.62 (+/-0.55)
4	0.66 (+/-0.15)	2.44 (+/-0.23)	3.90 (+/-0.22)
5	0.69 (+/-0.24)	2.12 (+/-0.49)	3.72 (+/-0.44)
6	0.50 (+/-0.16)	1.80 (+/-0.54)	3.26 (+/-0.85)
Distal			

Figure 3.4: Sheep in harness for preliminary in vivo load measurement

Preoperatively, the transducers and forceplate were calibrated statically over the expected range of measurement using deadweights. Prior to the commencement of each postoperative test, the pinsites were inspected and cleaned with 70% alcohol, and the pins tightened to exceed the predetermined minimum values.

The measurement system was then calibrated to no-load settings by suspending the right hindlimb off the ground with the animal relaxed. During a typical test, data were obtained from about 12-15 steps on the forceplate.

Bending moments were recorded in the first few days from the fixator on the well-vascularised osteotomy, but thereafter a fault developed in the transducer and further recordings were deemed unreliable. Nonetheless these data were sufficient to indicate the requirements for the definitive fixation device; on the second postoperative day the mean bending moment (+/- standard deviation) was 24 (+/- 2) Nm, and the maximum recorded in all tests was 39 Nm.

The axial load and ground reaction force were monitored in both animals over a period of four weeks after osteotomy (Tables 3.2 and 3.3). The persistently higher values in the fixator of the devascularised animal seemed to indicate a delay in healing (Table 3.2) which was also evident radiographically (Figure 3.5). Interpretation of these results was limited by the size of the experiment and the development of a pin track infection in the distal pin group of the well vascularised animal, as evidenced by the lower ground reaction forces measured on the fourteenth postoperative day (Table 3.3).

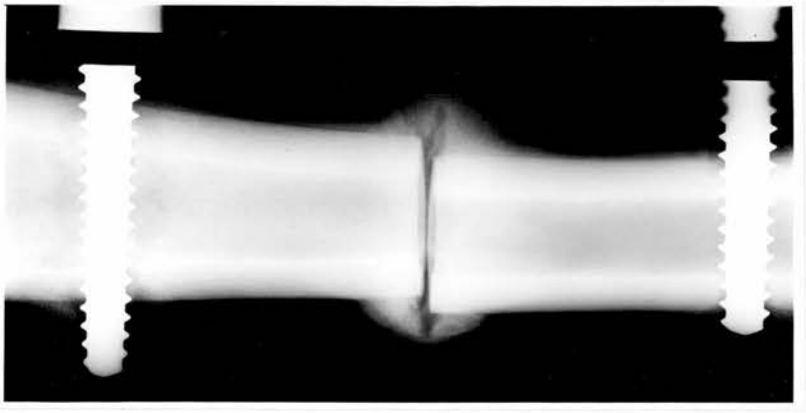
Table 3.2: Preliminary study: fixator axial loads (N)

Postoperative Day	Well-vascularised	Devascularised
2	341 (+/-38)	177 (+/-60)
7	258 (+/-56)	387 (+/-147)
14	108 (+/-28)	291 (+/-80)
21	82 (+/-11)	184 (+/-35)
28	58 (+/-32)	100 (+/-18)

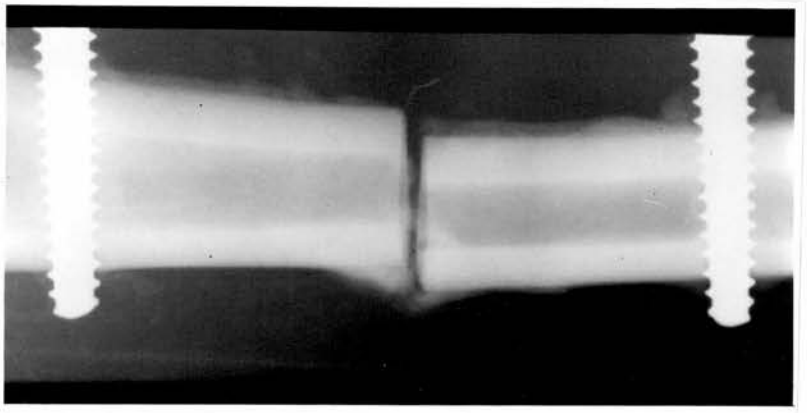
Table 3.3: Preliminary study: ground reaction force (N)

Postoperative Day	Well-vascularised	Devascularised
Preoperative	262 (+/-57)	226 (+/-65)
2	131 (+/-21)	83 (+/-31)
7	110 (+/-25)	127 (+/-57)
14	68 (+/-6)	110 (+/-36)
21	79 (+/-26)	95 (+/-27)
28	99 (+/-23)	137 (+/-37)

Figure 3.5: Preliminary study: radiographs at 28 days



Well-vascularised



Devascularised

3.3 Definitive experimental system

3.3.1 Variable axial stiffness external fixation device

The preliminary studies enabled the construction of a unilateral fixator composed of two pin holding blocks mounted upon linear bearings (Figure 3.6). The bearings each incorporated a stainless steel ball race which allowed the bearing to move freely along the steel track parallel to the long axis of the tibia. With the fixator and pins fully assembled, displacement at the osteotomy site in planes other than the long axis was estimated to be less than 1 mm for loads of 1000 N.

Relative movement of the pin blocks, and hence of the osteotomy fragments to which they were attached using the modified pins, was determined by a removeable module containing a spring of prescribed stiffness and a transducer capable of measuring the axial load in the fixator when the tibia was loaded during weightbearing.

3.3.2 Instrumented treadmill

A number of problems were encountered with the walking circuit used in the preliminary study. Although the animals learned to walk fairly quickly, only one step could be recorded on the forceplate with each revolution of the circuit, and occasionally the animal missed the forceplate altogether. In order to increase the number of steps collected at each in vivo measurement session and to standardise the velocity of the gait cycle in the sheep, a treadmill was specially constructed (Figure 3.7). The forceplate was incorporated in the floor of the treadmill, under the right rear quadrant of the moving belt. A programmable, variable speed electric motor drove the front roller of the treadmill.

Figure 3.6: Definitive experimental fixation device

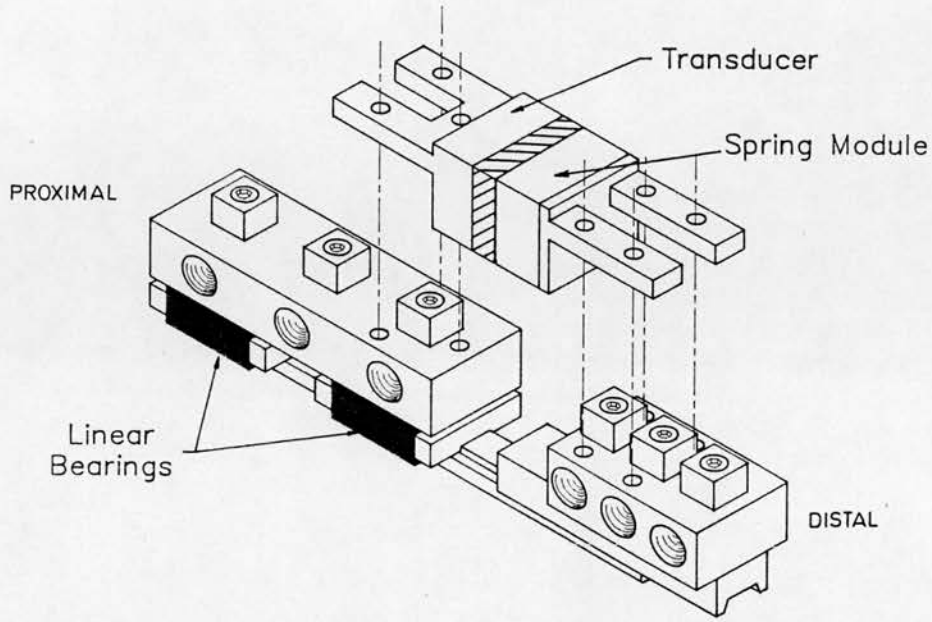
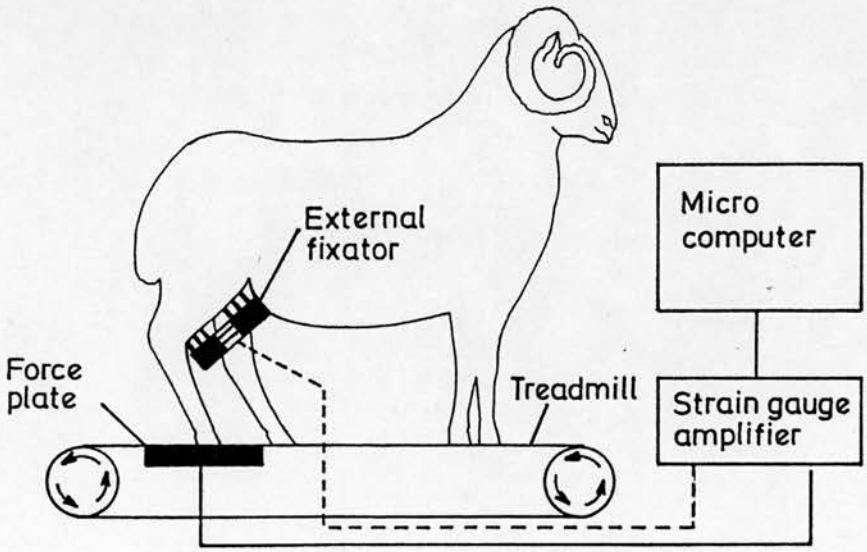


Figure 3.7: Instrumented treadmill apparatus



Use of the treadmill reduced the signal error due to electrical noise that proved to be a problem with the radiotransmission in the preliminary study. The fixator and forceplate were calibrated in the same manner employed in the preliminary study, although signals from the fixator bar were relayed via a flying lead directly to the microcomputer while the animal was on the treadmill. Although the relevance of treadmill gait to normal walking has been disputed, it has been shown that surface strains in the intact equine metacarpal while on a treadmill are equivalent in magnitude and orientation to those recorded during normal racetrack activity (Gross, McLeod and Rubin 1990). In any event the statistical advantages were considered to far outweigh any minor disturbances in gait pattern, the assessment of which was not the main object of the study.

3.3.3 Data collection, storage and retrieval

From the measurements taken, the parameters assessed were the number of steps, axial load, ground reaction force and stance phase duration. An editing programme allowed the operator to review the steps immediately following each test, excluding from statistical analysis those steps which were readily identifiable as abnormal; i.e. when the opposite limb was on the forceplate or when the osteotomised limb was only partially on the forceplate; these were determined from the shape of the stance phase curve, from ground reaction force values, and from the temporal relationship of the axial load signal to the ground reaction force signal. Edited data were then stored along with the raw test data and individual test statistics on floppy disks.

Axial interfragmentary strain was calculated from the measured axial loads and the measured in vitro stiffness of the fixator system (spring, bar and pins). Since the load and stiffness were measured, displacement at the osteotomy site could be calculated; this was then expressed as a percentage of the radiographic measurement of the osteotomy gap to

give axial strain.

3.4 Summary

The purpose of this investigation was not to analyse the effects of external fixation on fracture healing. However, it was appreciated that the external fixator provided the most readily adaptable method of controlling and measuring the relative displacement of the osteotomy fragments in order to observe a biological response with minimal direct interference from the fixation itself.

Preliminary studies provided the design specifications to construct a definitive fixator which enabled a range of parameters of the mechanical loading history of the osteotomy to be measured. Devascularisation by suppression of the periosteal and medullary networks appeared to retard the healing response, in terms of consistently higher loads carried by the fixator.

4. EXPERIMENTAL DESIGN AND PROCEDURE

4.1 Introduction

From the literature review it was evident that after a fracture, the role of blood flow, in particular from the periosteal or extraosseous source of revascularisation, seemed to be a very critical factor in determining the outcome of the healing process. At the same time, the vast body of research into mechanical factors demonstrated the significant effect of fixation on the rate and pattern of healing in the well-vascularised setting, especially in relation to the generation of periosteal callus.

The relative importance of the 'biological' variable of blood flow and the 'mechanical' variable of interfragmentary motion has yet to be defined, although some authors have acknowledged the problem (Hulth 1980, Gustilo, Merkow and Templeman 1990). After an extensive search, no previous study investigating afferent blood flow appeared to have employed strict monitoring of the mechanical conditions of the healing fracture. Although haemodynamic responses have been quantified in studies of fixation rigidity (Williams et al 1987, Smith, Bronk and Kelly 1990), these have not always examined the specific contribution of medullary, periosteal or intracortical blood supplies nor demonstrated the effect of exclusion of these individually in a strictly defined mechanical environment.

To address this issue, a series of experiments was designed to test the hypothesis that blood flow and axial interfragmentary movement (expressed as strain) were equally important in determining the outcome of fracture healing. The preliminary studies suggested that a combined insult to the medullary and periosteal vascular networks might delay healing. As the periosteal system was considered to be the more important, in the main study it was decided to attempt to isolate the periosteal contribution entirely, leaving the medullary system relatively undamaged, apart from the effect of the transverse

osteotomy.

The timescale of assessment was also important, as if an inappropriate stage was chosen, the effect of a particular variable might have been missed. For this reason, the majority of quantitative measurements were made at two weeks after osteotomy, which appears to correspond to the peak of blood flow (Paradis and Kelly 1975, McCarthy and Hughes 1984), and at six weeks, which appears to correspond to the maximum quantity of periosteal callus in similar models of experimental fractures (Williams et al 1987, Aro et al 1990, Markel, Wikenheiser and Chao 1990). A final stage at twelve weeks was also planned, but because of evidence that the early effects of mechanical and biological variables were most significant, and also because of the limitations of time and cost, these were not carried out.

4.2 Experimental groups

As in the preliminary study, crossbred Scottish blackface sheep were used as experimental animals. Age and sex are known to affect the structural and material properties of bone (Carter and Spengler 1978, Natali and Meroi 1989), and therefore these were controlled for in the selection of animals. Entry to the study was restricted to mature female sheep, of approximately three years of age, with no previous known injury or illness. Maturity was checked preoperatively by dental inspection by a registered Veterinary Officer, and confirmed postoperatively by radiographic evidence of closure of the proximal tibial epiphyseal growth plate. In addition the hooves of all animals were inspected preoperatively for evidence of footrot or other abnormality which might affect gait and if found, the animals were excluded.

Once selected, the animals were allocated to one of four main groups in the study. Each

consecutive animal was allocated to a different group in strict rotation, thereby minimising error due to observer bias or due to acquired experience with the surgical and measurement techniques as the experiment was conducted. The two week experiments were all completed before the six week experiments had commenced, but the same method of allocation was followed. Six complete fixation devices and measurement systems were constructed which determined the total number of animals which were studied at any one time. The experimental groups and relevant sample numbers are summarised in Table 4.1.

4.2.1 Control group

Although a unilateral osteotomy was the basis of the model, and therefore the contralateral tibia of each animal could serve as an 'internal' control for each individual animal, the possibility of an effect on the 'normal' contralateral limb as a consequence of the surgery or postoperative management was considered. In addition, there were no quantitative data in the literature on such aspects as the distribution of regional blood flow in or the mechanical properties of the tibia of the normal sheep. Therefore, a group of six normal sheep who had no osteotomy, fixation or other procedure served as a baseline control (Group N).

4.2.2 Standard group

The object of this group was to establish a well-vascularised osteotomy held in an external fixator with an axial stiffness equivalent to that of fixators used in clinical practice. An axial stiffness of 240 N/mm was selected based upon the in vitro comparative studies of Kempson and Campbell (1981), and this is approximately the stiffness of a bilateral Hoffmann configuration applied to a human tibia. This amount of stiffness was

determined in the definitive experimental fixator by the use of a silicone rubber block and epoxy resin spacer in the removeable module. The stiffness of the wholly assembled fixator, applied with six modified 10 mm pins at an offset distance of 60 mm from two wooden blocks representing the osteotomy fragments was tested in vitro using deadweights, with a horizontal dial test indicator (Mitutoyo UK Ltd, Andover, England) mounted within the gap to measure the resulting displacements (Figure 4.1).

Two subgroups were created in this group. The largest (Group S) underwent an osteotomy with careful protection of the periosteum and soft tissues, and were assessed at two *(n=8)* and six *(n=6)* weeks. Another group of three sheep (Group F) had the external fixator applied and a 'sham' incision but no osteotomy was performed, in order to determine the effect of the fixation alone on blood flow and biomechanical measurements, which was assessed at two weeks after surgery.

4.2.3 Rigid group

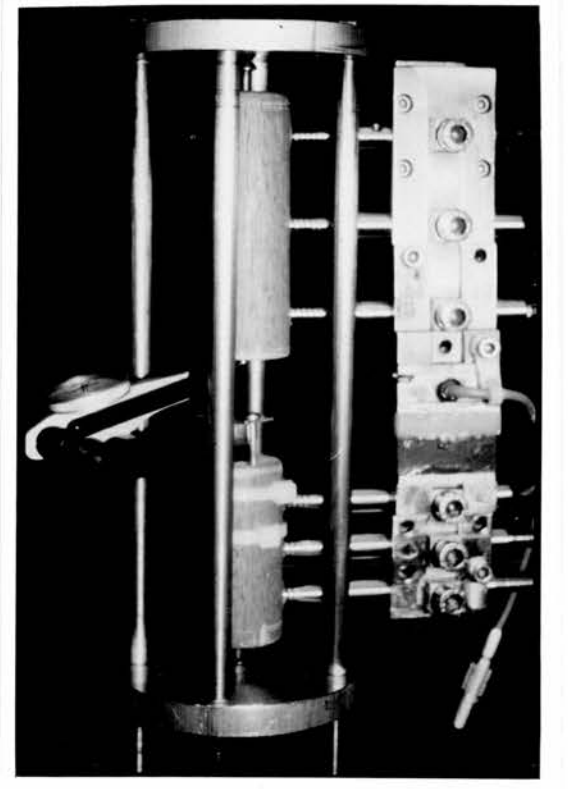
This group (Group R) was established to document the effect of fixation with higher axial stiffness, and hence potentially lower axial strain, in a well-vascularised environment. An axial stiffness of 480 N/mm was nominated which was approximately double that used in the standard group, and this was to be achieved by the use of a stainless steel block in the removeable module of the fixator. This was also tested *in vitro* using deadweights in the mock configuration. Five animals were assessed at two weeks, and five animals at six weeks.

Table 4.1: Experimental groups and numbers of animals

N: Normal (n=6)

	Two Weeks			Six Weeks		
Group	Initial	Exclusions	Final	Initial	Exclusions	Final
F	3	-	3	-	-	-
S	8	3	5	6	1	5
R	5	-	5	5	-	5
D	7	-	7	6	-	6
Total	23	3	20	17	1	16

Figure 4.1: In vitro calibration of axial stiffness



4.2.4 Devascularised group

The object of this group (Group D) was to quantify the effect of elimination of the blood supply to the osteotomy region from the periosteal and muscular circulation. The axial stiffness of fixation was 240 N/mm, the same as the standard group (Group S). Seven animals were assessed at two weeks, and six at six weeks.

4.3 Experimental protocol

4.3.1 Preoperative management

After selection the sheep were shorn, weighed and kept indoors in groups of six in straw-lined pens which were approximately twelve to sixteen square metres in area. The temperature was controlled at around 20 degrees Celsius. They were fed hay and a supplementary protein feed and were allowed to adjust to the surroundings for two weeks before surgery. In the week before surgery each sheep was taken to a room containing the instrumented treadmill and allowed to stand quietly on the belt for a few minutes. The sheep was then blindfolded and the treadmill started at low speed; a handle was installed on the front roller to assist with the initial steps until the sheep had the confidence to walk alone. Blindfolding was found to greatly assist the test procedure by reducing distractions and discouraging the animals from attempting to jump out of the treadmill.

Once accustomed to the treadmill, the sheep were walked every second day until surgery. On the morning of the operation, a final preoperative walk was performed, and a test recorded from the forceplate of the vertical component of the ground reaction force. For all tests the treadmill motor was programmed to run at a constant speed, which resulted in an average gait velocity of about 0.5 metres/second, equivalent to a slow walk. Typically

100-120 steps obtained in about three minutes were analysed.

4.3.2 Anaesthesia

The anaesthetic procedure was the same as that used in the preliminary study, i.e. induction and maintenance with a mixture of halothane, nitrous oxide and oxygen. This combination is widely used in veterinary practice (Hall and Clarke 1983). Halothane is a volatile anaesthetic agent which may cause cardiac and respiratory depression, resulting in lower cardiac output, lower arterial pressure and peripheral vasodilatation (British National Formulary, 1991). Specific effects of halothane on bone blood flow are not well described, although barbiturates have been shown to be associated with a fall in cortical blood flow of 25% in anaesthesia lasting more than sixty minutes in the rabbit (Davis, Holloway and Pooley 1990).

Every animal was intubated with a cuffed endotracheal tube because of the problems associated with regurgitation, which is a particular risk in ruminants such as sheep, especially when recumbent supine on the operating table. Ruminal tympany was reduced by starving the animals for 12 hours prior to surgery, and by slightly elevating the caudal end of the table to allow oesophageal contents to drain out during the operation. Operating times were typically one hour for the primary procedure and one and a half hours for the terminal procedure. Recovery from the primary procedure was usually rapid (within 30 minutes) when the above regimen was used.

For minor procedures such as radiographs and for certain injections during the experiment, a brief anaesthetic lasting only a few minutes was given using the same agents given through a full face mask, without intubation.

4.3.3 Primary surgical procedures

After intubation an intravenous cannula was inserted into the right jugular vein and a prophylactic dose of 1.5 g cefuroxime (Zinacef, Glaxo Laboratories Ltd, Greenford, Middlesex, England) a broad spectrum cephalosporin antibiotic, was given. The right hindlimb was then prepared in the same manner as described in the preliminary study, and the pins and fixator applied using aseptic technique to the anteromedial subcutaneous border of the tibia in the anteroposterior plane.

In Group F, a longitudinal incision was made between the two sets of pins. The incision was deepened through the deep fascia but the periosteum was not incised. The wound was then closed with an absorbable Vicryl suture (Ethicon Ltd, Edinburgh, Scotland).

In Groups S and R, the same exposure was performed but the periosteum was incised transversely, and a Gigli saw passed behind the tibia. An osteotomy was then made leaving a 2 mm gap, and the saw debris removed. The incision was then closed.

In Group D, following the osteotomy, the fixator was disassembled by removing four screws on the track of the linear bearings. This allowed the osteotomy fragments to be manipulated to enable complete circumferential stripping of the periosteal membrane from the underlying cortex for 20 mm from the osteotomy in each fragment (Figure 4.2). A 40 mm silicone rubber sleeve made from standard laboratory tubing, with an internal diameter of 12.5 mm and a wall thickness of 1.25 mm (Mackay and Lynn, Edinburgh, Scotland) was then placed across the osteotomy, enclosing the bone ends. The osteotomy was then reduced and the fixator reassembled to form a 2 mm gap. The purpose of the subperiosteal sleeve was to prevent revascularisation from either the periosteum or the adjacent muscle. The muscles themselves were not injured by this procedure and their extraperiosteal connections were left intact. The incision was then closed, as in the other groups.

Figure 4.2: Technique of periosteal stripping

Figure 4.3: Measurement of pin torque

After the osteotomy in each animal, the broad spectrum antibiotic and bone-seeking fluorochrome oxytetracycline (Terramycin, Pfizer Ltd, Sandwich, Kent, England) was given in a dose of 25 mg/kg as a slow intravenous injection over 30 seconds into the jugular vein. Following closure, the wounds and pinsites were dressed with cotton gauze swabs soaked in 70% alcohol. The grub screws on each individual bone pin were then released in turn and the torques measured to ensure they were above the predetermined thresholds (Figure 4.3). The fixator and limb were then wrapped extensively with cotton wool and a 'Vetrap' self-adhesive bandage applied (3M Company Inc, Minneapolis, Minnesota, USA).

4.3.4 Postoperative regimen

During the early postoperative period a regular schedule of analgesia and antibiosis was followed in every animal. Intramuscular injection of buprenorphine hydrochloride, an opioid analgesic (Temgesic, Reckitt and Colman, Hull, England) in a dose of 0.6 mg every 4-6 hours was sufficient to reduce subjective observations of recognised signs of pain such as tachypnoea.

Cefuroxime was given in an intramuscular dose of 750 mg daily for three days in an effort to prevent serious wound or pin track infection. In the animals observed for the six-week period, further fluorochromes were administered. At two weeks, an intravenous dose of 10 mg/kg calcein (Sigma Chemical Company, St Louis, Missouri, USA) was given, followed at four weeks by 50 mg/kg xylenol orange (Sigma Chemical Company). Both these fluorochromes were mixed with 20 mg/kg sodium bicarbonate, in accordance with recommended doses (Plenk 1986).

At two and four weeks routine radiographs in the lateral view were taken under a brief general anaesthetic with the animal in the semiprone position. The x-ray plate, exposure

and distance of the tube from the leg were standardised. The measurement of axial strain, mechanical properties, blood flow and mineral uptake both in vivo and at the terminal procedures are described in detail in the succeeding chapters of this thesis.

4.4 Statistical methods

The majority of observations made in this study were quantitative. The outcome measures of applied in vivo loads, radiographs, blood flow, mineral uptake, torsional properties and histomorphometry were compared between groups by using parametric methods of analysis. Since in most of the individual group results the standard deviations were approximately proportional to the arithmetic means, and it is an underlying assumption of most parametric tests that the observed values fit the normal distribution, all raw data was transformed to logarithmic values to allow meaningful application of standard tests (Kirkwood 1988).

Differences between means in each of the three main groups (S, R and D) were first tested using a one-way analysis of variance; if significant differences were found, these were identified using the unpaired Student's t-test. In these cases comparisons were made directly between Group S and Group R, or between Group S and Group D; in other words, Group S acted as a control for both Group R and Group D, which corresponded to variations in mechanical environment or afferent blood flow, respectively. Direct comparisons could not be made between Group R and Group D because there were inherent differences in both blood flow and fixation rigidity as a consequence of the experimental design. Differences between right and left limbs within each group were tested using the paired t-test.

In this study, a result was considered significant if the probability of the difference occurring due to chance was less than 5% ($p<0.05$). Although the tests of significance

were carried out on the logarithmic values, for reasons of clarity the original non-transformed arithmetic means and standard deviations have been presented in preference to the geometric means and confidence limits.

All quantitative data was entered into a series of databases set up using dBase III Plus database software (Ashton-Tate Inc.) which was run and stored on an IBM-compatible PC microcomputer. Statistical calculations were performed with the dBase STATS package (SPSS Inc.) which was linked to the database software.

4.5 Complications and exclusions

4.5.1 Inadequate fixation

The fixation system proved to be very robust and there were no major displacements due to material failure of the components of the system. However, in two of the two week sheep in Group S, corrosion of the linear bearings due to the chemical action of a povidone-iodine antiseptic (Betadine, Napp Laboratories, Cambridge, England) resulted in jamming of the axial movement of the fixator. In one six week animal, also in Group S, the secondmost proximal pin failed to engage the far cortex when inserted at operation, despite achieving a satisfactory torque. The proximal fragment was therefore mainly held in solid cortical bone by the third most proximal pin, the first pin having been inserted in the thin cortex of the metaphysis. The correspondingly greater stresses on the third pin resulted in significant bending, sufficient to cause narrowing and tapering of the osteotomy gap and this was visible radiographically by the second postoperative week (Figure 4.4). These three animals were excluded from statistical analysis.

4.5.2 Fracture

One sheep in Group S, which was assessed at two weeks, sustained a fracture through the distal pin group during the first postoperative week while ambulant in the pen (Figure 4.5). Although the gap appeared to be maintained, the loading conditions were undoubtedly different and because of considerable limping further *in vivo* analysis was discontinued.

4.5.3 Involucra

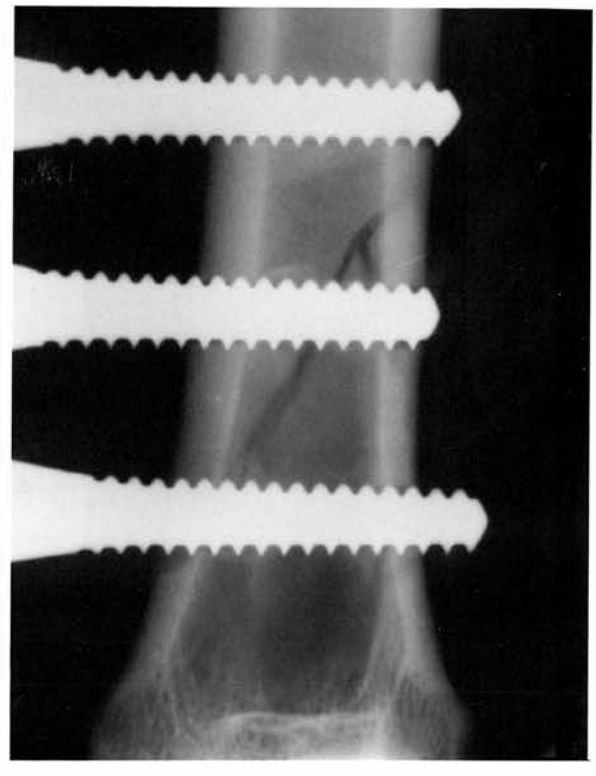
The formation of subperiosteal new bone around the outside of the silicone rubber sleeve occurred to some extent in all the sheep in Group D. However, despite being prominent radiographically (see Figure 5.4) in no animal did it form a complete bony bridge around the sleeve, as assessed under direct vision post-mortem. Therefore the calculated axial strains in the six week group were taken to reflect those actually occurring at the osteotomy site, within the sleeve. Accordingly, all the animals in this group were included in the statistical analysis.

4.5.4 Pin track infection

The dressings around the limb and each individual pin were changed weekly in all groups. On each occasion the pins were inspected and a note made of the presence of any swelling, discharge or obviously purulent infection. In addition, each pin track (including the medullary canal) was swabbed within two hours of death when the pin was removed after mechanical testing; these samples were placed in clear transport medium (Transwab, Medical Wire & Equipment Co Ltd, Corsham, Wilts., England) refrigerated overnight at 4 degrees Celsius and sent for microbiological culture.

Figure 4.4: Inadequate fixation

Figure 4.5: Distal pin group fracture



This survey was conducted at two and six weeks, no distinction was made between groups and the results were pooled for analysis. Overt clinical pin track infection, with swelling, erythema and purulent discharge was found in 4 sheep (10%) involving a total of 6 pins (2.5%) and occurred mainly in the distal pin group where the pins were closest together. Minor haemoserous discharge was noted from a similar number of pinsites. The remainder of the pinsites were dry but 75% yielded a positive growth on culture (Table 4.2), mainly of skin or bowel commensal organisms.

These data are in general agreement with the canine model of pin track infection used by Respet, Kleinman and Meinhard (1987). They found positive cultures from the medullary canal of 50% of pinsites after two weeks, which rose to 67% after four weeks, and these were related to the skin flora in the majority of cases. Although they did not use a fracture model, and the pins in their study were not loaded, it is reasonable to conclude from both studies that colonisation of pin tracks is a common occurrence, but that other factors must explain the development of overt clinical infection.

4.5.5 Pin loosening

The importance of an efficient mechanical link at the bone-pin interface in the interpretation of axial load measurements from the bar of an external fixator has already been described. Before each weekly treadmill test, the torque of each pin was measured with the calibrated torque wrench, and if less than the predetermined thresholds, tightened. Because of this procedure, the 'natural' course of decay of the pin torque could not be assessed; however, the tendency to loosen may be inferred in this study from the frequency of tightening required to reach the predetermined thresholds, and this was recorded for every pin on a weekly basis. It is possible that the tendency to loosen may

Table 4.2: Incidence of pin track infection

	Pins	%
Total pins used in 40 sheep:	240	100
- clinical infection	6	2.5
- haemoserous discharge	6	2.5
Total pin sites cultured:	204	85
- sterile	51	25
- positive isolates	153	75

Table 4.3: Frequency of pin tightening per sheep

Pin	Standard	Rigid	Devascularised
Proximal			
1	2.7	3.6	3.6
2	1.8	2.7	1.7
3	1.4	2.5	1.4
4	1.0	1.2	1.5
5	1.5	2.7	1.6
6	2.2	3.2	2.4
Distal			
Total	10.6	15.9	12.1

have been exacerbated by the process of repetitive pin adjustment itself, and therefore caution is required in extrapolating the findings to the clinical situation.

In all three osteotomised groups, the pins in the cortical bone of the mid-diaphysis required tightening the least number of times, in comparison with those pins in the metaphyseal regions which usually required tightening weekly. The pooled data for each group is presented in Table 4.3, which reveals that the pins in Group R required tightening 50% more frequently than those in Group S.

5. MEASUREMENT OF APPLIED LOADS AND RADIOGRAPHY

5.1 Introduction

During normal activity, long bones of the limbs such as the tibia are subject to a variety of loads. Generally these are either external loads which the limb must carry, such as the mass of the animal's body, or internal loads due to the muscles which act on the bones in the process of locomotion (Cappozzo, Figura and Marchetti 1976).

In the sheep, the tibia is inclined cranially at an angle of about 60 degrees from the horizontal; the femur and metatarsal bones are inclined caudally. Because of this arrangement, the limb is prevented from collapsing spontaneously under the weight of the animal by the muscles of the hindlimb, which must always be active to some extent while the animal is standing. Hence the tibia will be loaded by a number of muscles which will be acting in a complex of directions to counteract the effect of gravity on the mass of the animal.

The forces generated by the muscles on the tibia therefore present considerable difficulties in measurement. However, the external load may be measured in terms of the ground reaction force (GRF) using a forceplate. Since force is a vector quantity, this force may be resolved into vertical, horizontal transverse and horizontal longitudinal components, of which the vertical is greatest in magnitude in the quadruped during walking (Schamhardt and Merkens 1987).

Jayes and Alexander (1978) quantified the vertical component of the GRF in the sheep and found that during walking, the forelimb exhibited a single-peaked curve and carried proportionately more load than the hindlimb, which described a twin-peaked curve with a maximum value of about 40% body weight. This difference in GRF, which was found with all

gaits, was due to the centre of mass of the animal lying closer to the forelimbs. These findings were supported by Pandy et al (1988) who also showed that in both fore and hind limbs of the goat, absolute GRF values were found to increase with speed.

In the present study, the axial load measured from the fixator, from which the axial displacement and strain were calculated, represented the sum of the external loads and muscular loads acting on the osteotomised tibia in the axial direction. However the external loads differed considerably between animals, probably because of differences in weightbearing as a consequence of the healing process itself or other subjective and uncontrolled variables such as pain. To allow a more accurate interpretation of axial load, the vertical component of the ground reaction force was simultaneously monitored in each animal.

5.2 Treadmill test procedure

5.2.1 In vitro system stiffness

To account for the possibility of variation in the material properties of the silicone rubber used in Groups S, F and D the axial stiffness of each spring component was measured prior to assembly of the fixator and application to each animal. The axial stiffness was measured in compression on a tensile testing machine (Lloyd Instruments Ltd) and found to be linear over the range of loads measured in the preliminary study. The mean system stiffness (+/- standard deviation) for Group S was 238 (+/- 6) N/mm, and for Group D was 241 (+/- 8) N/mm, which was acceptably close to the chosen value of 240 N/mm for these groups. The material properties of the stainless steel blocks used as springs in Group R were assumed to be more consistent than the silicone rubber. The measured in vitro axial stiffness of 460 N/mm, taken to represent the axial stiffness of all the systems in Group

R, represented a 92% increase in fixation stiffness compared to Groups S and D.

5.2.2 Sampling

Preoperatively, a single treadmill test was carried out as described in Section 4.3.1. Postoperatively, tests were carried out on a weekly basis after the pins had been tightened to the predetermined thresholds. On each occasion, the number of individual steps occurring in a test lasting about three minutes was recorded and corrected after editing. The mean (+/- SD) number of steps per test, calculated from all tests in the six week period was 58 (+/- 22) steps in Group S, 49 (+/- 24) steps in Group R, and 54 (+/- 22) steps in Group D.

5.2.3 Measurement error

Possible systematic sources of error in the measurement system were identified to include signal noise due to electrical interference from the treadmill motor, non-linearity of the response from the transducers, and change in the material properties of the silicone rubber spring components with time or with load. However, these were able to be approximated in most cases, and the maximum systematic error in load measurement was estimated to be less than 5%. Random errors, due to slight variation in belt speed, or due to inaccurate determination of the no-load setting, could not be quantified for each animal but were also felt to be insignificant in comparison to the measured values.

5.3 Results of in vivo monitoring

The animals recovered well from the operative procedures and were ambulant from the first postoperative day. The treadmill tests were also tolerated without difficulty and by their brevity caused little apparent distress. A typical example of the computer recording from a single step is shown in Figure 5.1. Generally, a curve characterised by a rapid rise, followed by a broad plateau or rounded peak, and then a rapid fall was seen. The twin-peaked curve described by other investigators was only noted infrequently, but since most of these earlier studies relied instead on floor-mounted forceplates, a slightly different pattern of gait may explain this qualitative difference.

5.3.1 Ground reaction force

The results of the preoperative assessment are presented in Table 5.1. It can be seen that despite random selection of animals into the study, analysis of variance revealed a significant difference in the preoperative weights, expressed in Newtons ($F= 6.29$ $df= 5,24$ $p<0.005$). This difference was mainly due to Group R, which had an approximately 20% lower mean weight than the other two groups. As a consequence, the preoperative GRF values were also different, but the lower mean of 178 (+/- 35) N in Group R only just achieved statistical significance when compared to Group S ($p=0.048$). The ratio of GRF to preoperative weight was not different between groups and agrees well with the data of previous workers (Jayes and Alexander 1978, Pandy et al 1988). However, to account for any effect of the discrepancy in weight and allow a meaningful comparison between groups, the GRF recorded in the postoperative tests was normalised, i.e. expressed as a proportion of the preoperative weight, in Newtons, of each animal.

At seven days after osteotomy, the group with the most rigid fixation (Group R) showed the

Figure 5.1: Load curves from a single step

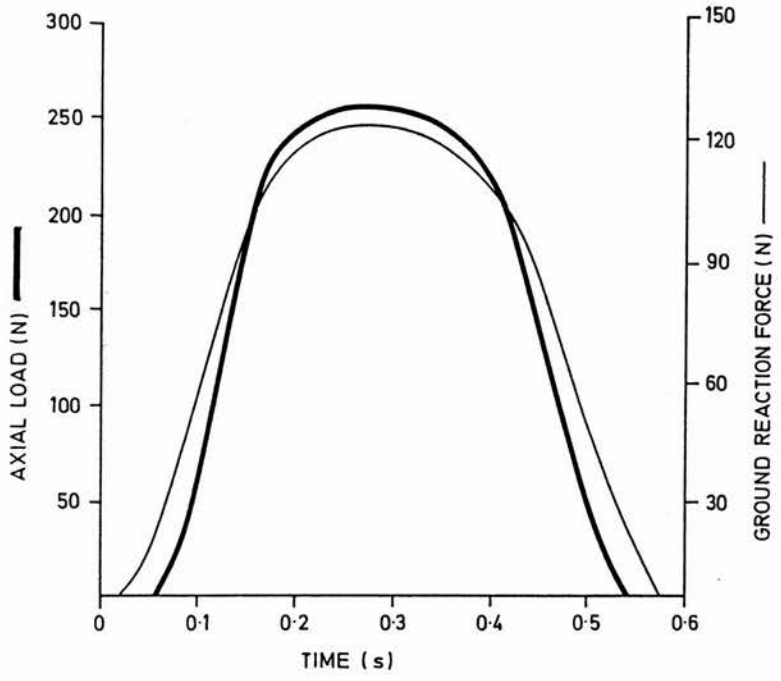


Table 5.1: Preoperative load analysis

	Rigid	Standard	Devascularised
Number of steps	61 (+/-20)	63 (+/-40)	62 (+/-29)
Weight (N)	436 (+/-53)[a]	536 (+/-62)[a]	511 (+/-91)
GRF (N)	178 (+/-35)[b]	207 (+/-49)[b]	196 (+/-71)
Stance phase (s)	0.60 (+/-0.06)	0.58 (+/-0.09)	0.61 (+/-0.11)
Mean GRF/mean weight	41%	39%	38%

highest GRF, which was significantly greater than the other two groups ($p<0.05$). In both well-vascularised groups there was a slight decrease in the succeeding weeks (Figure 5.2), but by the end of the experimental period both groups were weightbearing equally well to a level of over 30% body weight, which equates to about 75% of normal weightbearing for the hindlimb. Nonetheless the rigid group also had the greatest frequency of pin tightening (Table 4.3), which suggests that the additional external loads facilitated by the rigid fixation resulted in higher bone-pin interface stresses, deformation and subsequent loosening.

The devascularised group (Group D) showed initially similar ground reaction forces to Group S, but after 21 days weightbearing was significantly lower ($p<0.05$) and demonstrated a progressive decline until the conclusion of the experiment. At 42 days mean GRF was only 16.5% body weight, or approximately 40% of normal preoperative weightbearing. This was manifest clinically as a moderate but consistent limp, both within the pen and during the treadmill tests.

5.3.2 Stance phase time

The duration of the stance phase, analogous to the period between 'heel strike' and 'toe off' in the gait of the human subject, was also measured. There were no differences preoperatively between groups in terms of the contact time of the hoof upon the forceplate during each step, which averaged 0.6 seconds.

Postoperatively, the pattern of results was closely allied to that of ground reaction force (Table 5.2). In the early phase Group R showed longer mean stance phase times than the other groups, although this did not reach statistical significance. In the later part of the experiment, as expected, there were significantly lower stance phase times for

Figure 5.2: Normalised ground reaction force

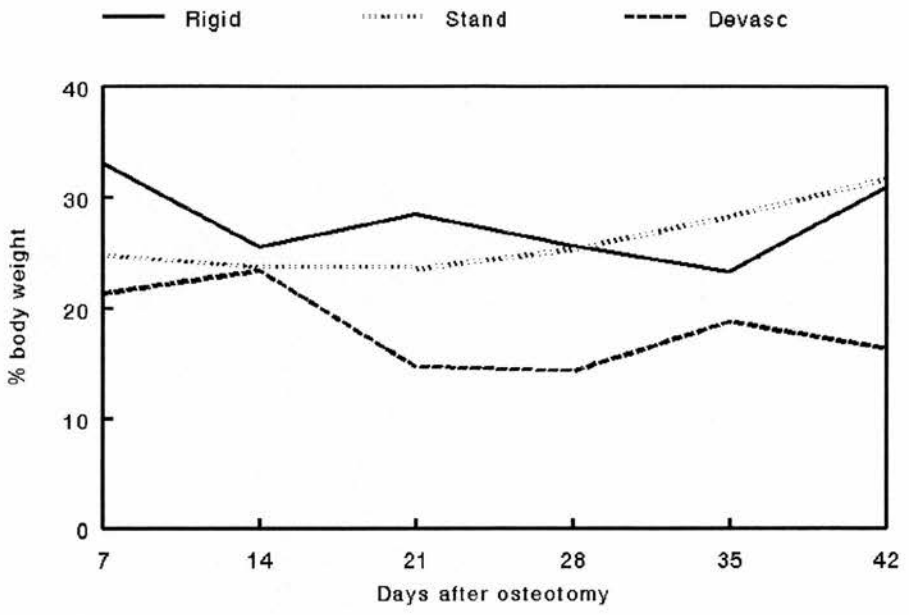


Table 5.2: Stance phase time (s)

Postoperative Day	Rigid	Standard	Devascularised
7	0.65 (+/-0.29)	0.46 (+/-0.14)	0.39 (+/-0.17)
14	0.47 (+/-0.10)	0.46 (+/-0.12)	0.41 (+/-0.18)
21	0.40 (+/-0.06)	0.51 (+/-0.13)[a]	0.32 (+/-0.08)[a]
28	0.42 (+/-0.08)	0.49 (+/-0.09)[b]	0.28 (+/-0.08)[b]
35	0.41 (+/-0.06)	0.52 (+/-0.10)[c]	0.37 (+/-0.12)[c]
42	0.47 (+/-0.11)	0.54 (+/-0.12)[d]	0.35 (+/-0.13)[d]

Group D ($p<0.01$), which together with the GRF results indicated that the animals with devascularised osteotomies were attempting to reduce both the quantity and duration of the applied external load on the operated limb.

5.3.3 Axial load, displacement and strain

The load on the transducer in the longitudinal axis of the fixator was the primary measurement in the determination of osteotomy gap strains. However there are several important assumptions which must be articulated before a valid interpretation of the results for osteotomy displacement and strain can be made.

Firstly, the axial load transducer was calibrated from a no-load setting prior to the commencement of each test, achieved by suspending the right hindlimb off the floor with the animal as relaxed as possible. However 'zero' load was not necessarily accurate, as even in this situation there was likely to be a small muscular component acting on the osteotomised tibia, and hence the fixator. This would have the effect of tending to underestimate the measured axial load.

Secondly, the calculation of axial displacement requires the measured axial stiffness of the fixator construct, which was only measured in vitro using wooden blocks as mock bones. However, it was reasonable to assume that it remained constant for each fixator throughout the experiment. The linear bearings were examined for movement immediately prior to each test and a valid test recorded only if there was no evidence of jamming.

Thirdly, the calculation of strain was reliant upon the accurate determination of the axial dimension of the osteotomy gap prior to loading. While it was not possible to directly measure this in vivo on the occasion of each test, and although radiographic

evidence suggested that in all groups the original osteotomy line was still present at the end of the experiment, it is arguable that from soon after the operative procedure there would be some tissue present in the gap itself which would therefore have the effect of reducing the actual gap size prior to loading. Because of the difficulty in estimating the extent of this tissue, and in order to allow comparison from week to week and between groups, the calculated displacement at each week was divided by the standardised gap dimension of 2 millimetres throughout the study.

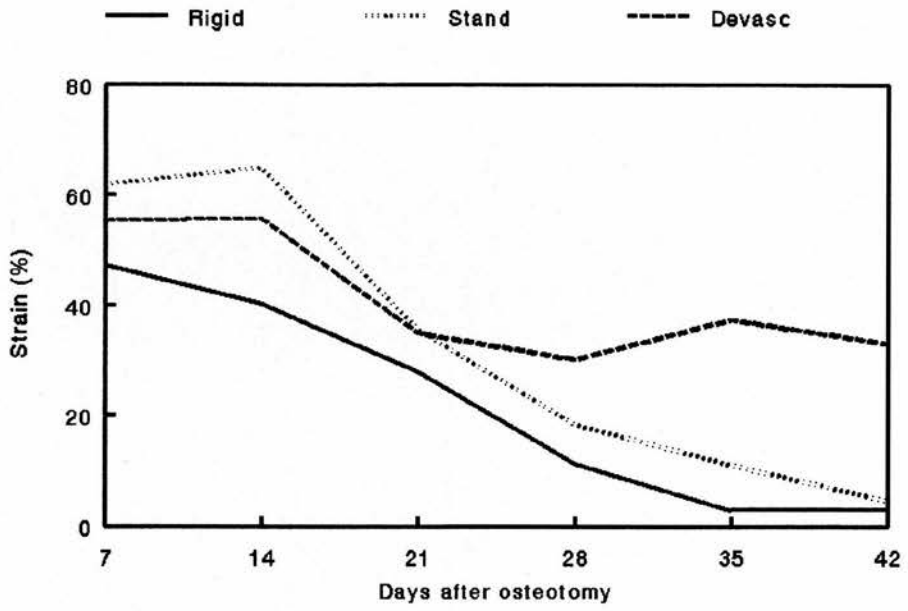
Analysis of variance of the axial load measurements suggested that there were significant differences in both the early (7 and 14 days) and late (35 and 42 days) phases of the experiment. The fixator axial load and calculated osteotomy displacements are given in Table 5.3 while axial gap strains are demonstrated graphically in Figure 5.3. The rigid fixation in Group R carried the highest axial loads at seven days after osteotomy, roughly equivalent in magnitude to the mean preoperative body weight for that group, which was significant ($p<0.05$). The measured axial loads corresponded to approximately double the raw GRF values, the difference probably being due to the considerable internal muscle loads sustained by the ovine tibia.

In terms of osteotomy displacement and strain, however, Group S had significantly higher values than Group R at fourteen days ($p<0.05$). This indicated that despite the initially lower axial loads imposed on the fixator, the difference in fixation stiffness was sufficient to ensure significantly greater micromovement at the osteotomy site, of the order of 1.3 mm compared to 0.81 mm, in the well-vascularised groups. Expressed as strain, this was 65% in Group S and 40% in Group R at the two-week stage. In the middle phase of the experiment (21-35 days), the linear reduction of strain was the same in both well-vascularised groups at a rate of approximately 1.8% per day. By six weeks mean strains were less than 5%, the fixator was sustaining less than 30 N axial load, and mean displacements were no greater a tenth of a millimetre in both groups.

Table 5.3: Fixator axial load and osteotomy displacement

Postoperative Day	Rigid	Standard	Devascularised
Axial load (N)			
7	438 (+/-144)[a]	297 (+/-92)[a]	270 (+/-132)
14	371 (+/-115)	314 (+/-128)	273 (+/-148)
21	259 (+/-143)	169 (+/-98)	169 (+/-83)
28	107 (+/-51)	87 (+/-86)	143 (+/-59)
35	29 (+/-18)	52 (+/-52)[b]	178 (+/-104)[b]
42	28 (+/-32)	23 (+/-20)[c]	155 (+/-95)[c]
Displacement (mm)			
7	0.95 (+/-0.31)	1.24 (+/-0.39)	1.11 (+/-0.53)
14	0.81 (+/-0.25)[d]	1.30 (+/-0.53)[d]	1.12 (+/-0.60)
21	0.56 (+/-0.31)	0.71 (+/-0.42)	0.71 (+/-0.35)
28	0.23 (+/-0.11)	0.37 (+/-0.37)	0.61 (+/-0.26)
35	0.06 (+/-0.04)	0.22 (+/-0.22)[e]	0.75 (+/-0.43)[e]
42	0.06 (+/-0.07)	0.10 (+/-0.09)[f]	0.66 (+/-0.41)[f]

Figure 5.3: Calculated axial strain



In contrast, after fourteen days the devascularised group (Group D) showed no net reduction in axial load, displacement or strain. At 35 and 42 days these parameters were significantly different from Group S ($p<0.05$), consistent with a substantial delay in the healing response as a consequence of devascularisation. The relatively high axial loads carried by the fixator in the late phase of the experiment suggested the substantial lack of any structurally-competent tissue between the fragment ends.

5.4 Radiography

Plain radiographs were made in the lateral plane only at two and four weeks using the procedure described in Section 4.3.4. Radiographs in the anteroposterior plane were not contemplated because the fixator obscured the osteotomy site, and a technique for oblique views proved difficult to standardise. The final radiograph for each animal was made after the leg had been amputated following the terminal procedure at six weeks. In this instance, the tibia, with the fixator still in situ but with the soft tissues removed, was placed directly in contact with the film (Singul-X RP medical x-ray film, CEA AB, Strangnas, Sweden) and exposed for five minutes in a Faxitron x-ray machine (Hewlett-Packard Inc). This enabled an image to be acquired with a higher resolution than that obtainable with the standard in vivo technique.

5.4.1 Qualitative radiographic findings

Comparative images at two, four and six weeks are shown in Figure 5.4. Typically, the first evidence of radiographic new bone formation was seen in Group S at two weeks as two small fronts of mineralised tissue forming on the periosteal surface on the side closest to the fixator; this phenomenon was less evident in Group R. In Group D there was no

discernable reaction at the osteotomy site, although crests of periosteal new bone were seen at the proximal and distal margins of the silicone rubber sleeve where the periosteum had been stripped back.

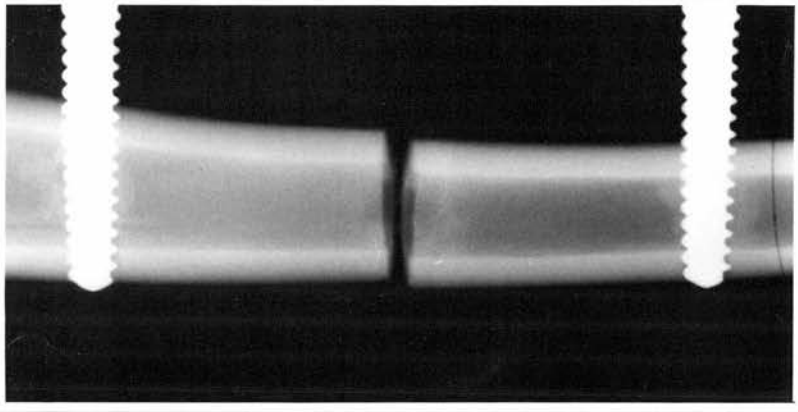
At four weeks after osteotomy, the most abundant callus response was again observed in the standard group which had recorded the highest displacements and strains during the treadmill tests. The callus fronts were visible forming a prominent arch which often joined at a considerable distance from the periosteal surface, enclosing a radiolucent area extending toward the osteotomy. A similar pattern was seen in the rigid group, but the callus bridge was smaller and joined in closer proximity to the original cortex. There was little evidence of medullary callus formation in either of the well-vascularised groups. On the other hand the devascularised osteotomies consistently demonstrated a degree of endosteal cortical erosion which was greatest at the proximal margin of the silicone sleeve, tapering distally to the original cortical thickness at the osteotomy site.

By six weeks the well-vascularised osteotomies were radiographically united by periosteal callus, although the osteotomy gap remained clearly visible. The gap itself was crossed by mineralised tissue, although this appearance may have been due to superimposition of the periosteal callus. Group S maintained the consistently larger area of callus seen at the earlier stages when compared to Group R. In Group D, endosteal resorption of the proximal fragment was much advanced, but only infrequently was resorption in the distal fragment seen. Significantly in three of the six animals in Group D assessed at six weeks, fine trabeculae of medullary new bone were observed crossing the osteotomy gap, in the absence of any activity on the periosteal surface.

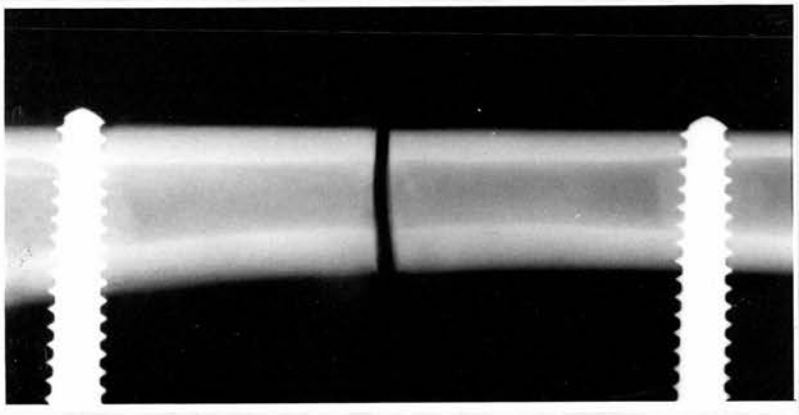
Figure 5.4: Serial radiography: (i) 14 days

Proximal *Distal*

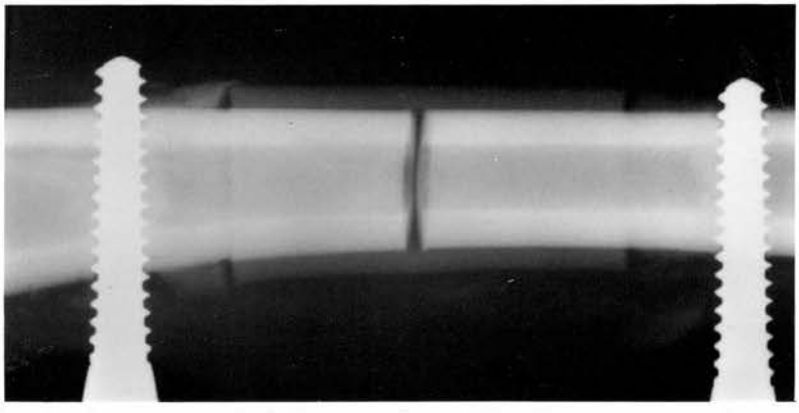
Rigid



Standard



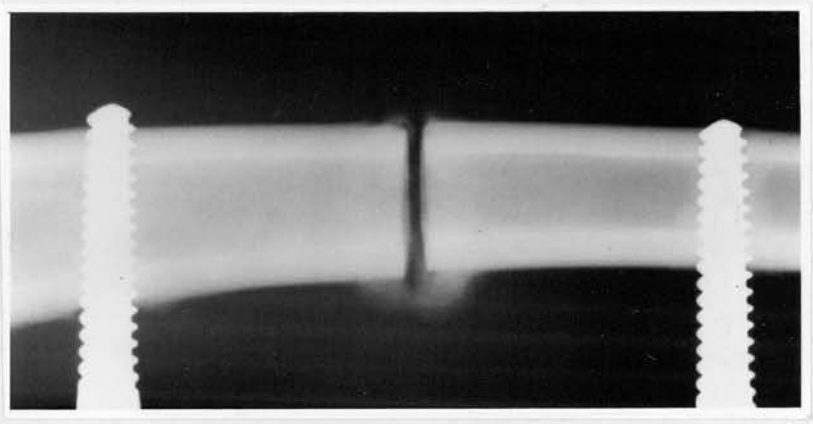
Devascularised



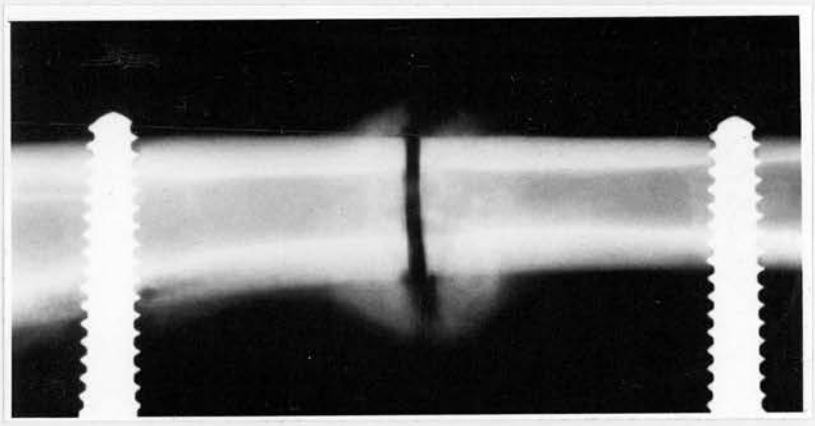
(ii) 28 days

Proximal *Distal*

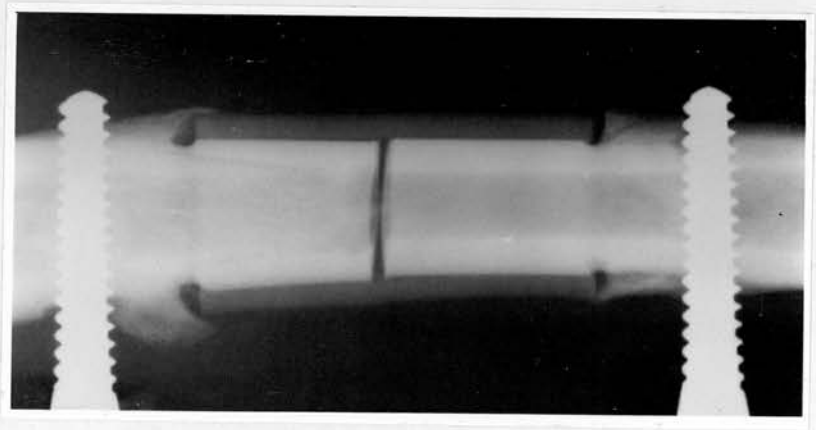
Rigid



Standard



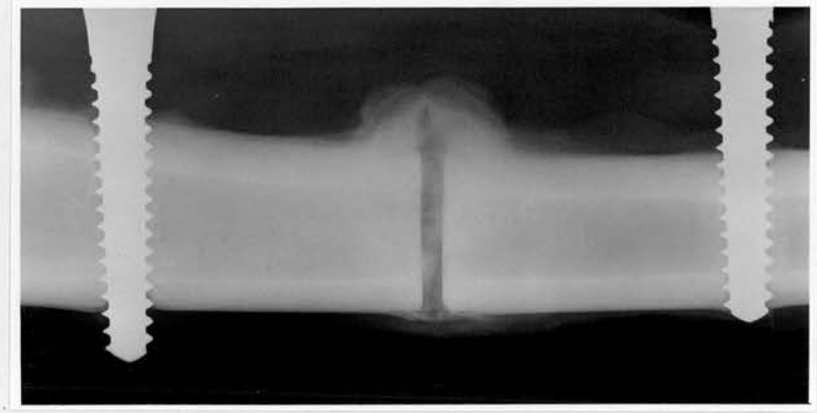
Devascularised



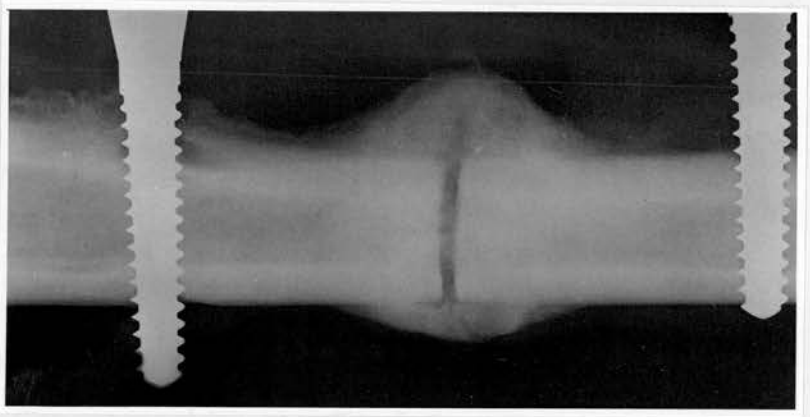
(iii) 42 days

Proximal *Distal*

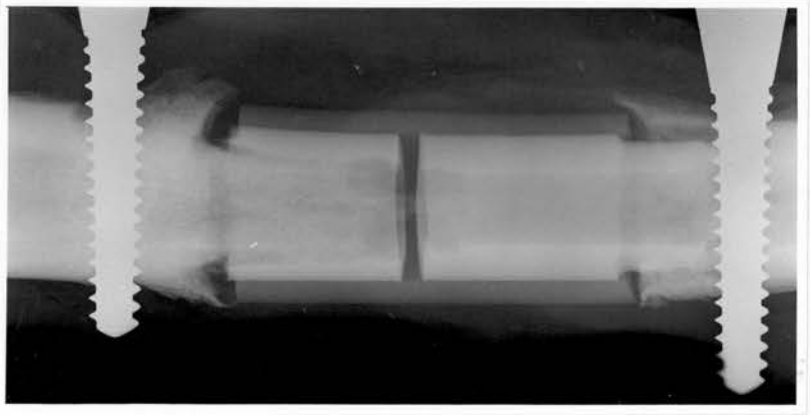
Rigid



Standard



Devascularised



Around the bone-pin connection with the near cortex a periosteal reaction was often seen, mainly affecting the pins closest to the osteotomy. Radiolucent resorption cavities around the tapered shank were also noted at some pinsites, but these were confined to the periosteal surface and did not appear to extend very far across the cortex.

5.4.2 Radiographic densitometry

On each lateral radiograph made at six weeks, quantitative profiles of radiographic 'density' were made perpendicular to the long axis of the tibia, using a computerised image analysis system (Magiscan, Joyce-Loebl Ltd, Gateshead, Tyne and Wear, England).

The radiographs were illuminated on a viewing box and an image of the osteotomy site captured by a video camera and displayed on a high-resolution monitor. A 64-level greyscale was automatically generated for each image and expressed in terms of pixel energy value (PEV). Profiles of PEV across the bone were made through the middle of the osteotomy gap, and at parallel planes two millimetres proximal (P) and distal (D) to the gap. At each plane two lines, each one pixel wide, were drawn and integrated to give a mean PEV profile. A plot of each profile was then produced as shown in Figure 5.5, and the area under each curve quantified.

In all groups the peak values and area under the curve (AUC) were lowest in the mid-osteotomy plane. In the planes adjacent to the gap, a bimodal pattern with a central plateau was usually observed, corresponding to the cortices and intermediary medullary callus. In the well-vascularised groups (Groups S and R) there was no significant difference between the AUC for proximal and distal planes within each group or when expressed as a P:D ratio, which had a mean value of 1.035 for both groups (Table 5.4). However the devascularised group had a significantly lower mean (+/- SD) P:D ratio of

Figure 5.5: Photodensitometric profile of rigid osteotomy

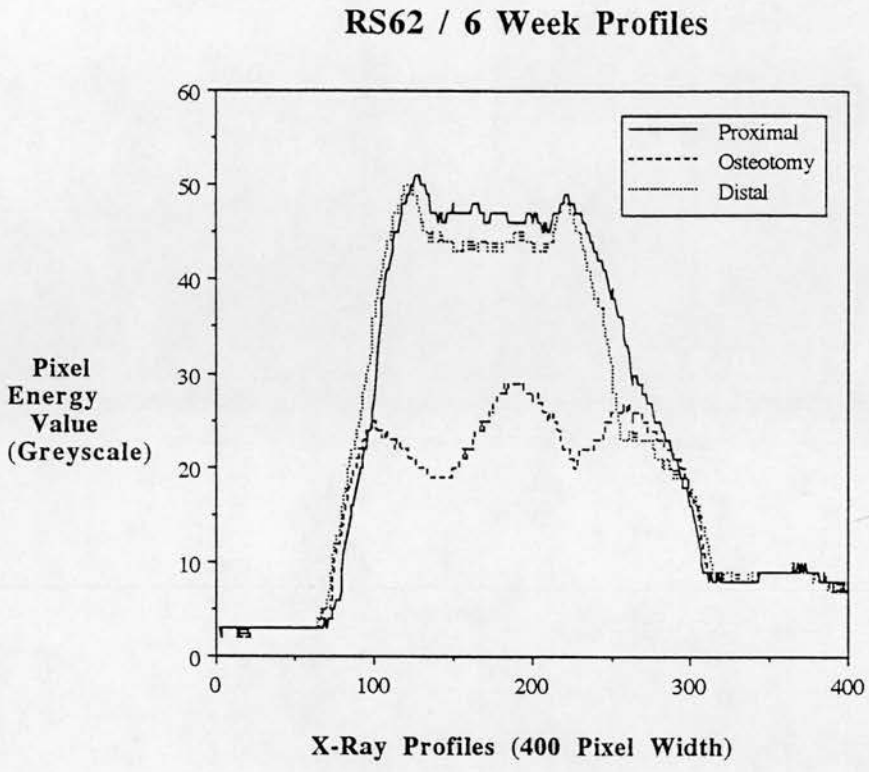


Table 5.4: Photodensitometric ratios

	Rigid	Standard	Devascularised
Proximal : Osteotomy	1.386 (+/-0.142)	1.641 (+/-0.333)	1.527 (+/-0.321)
Distal : Osteotomy	1.340 (+/-0.136)	1.579 (+/-0.252)	1.636 (+/-0.314)
Proximal : Distal	1.035 (+/-0.040)[a]	1.035 (+/-0.083)[b]	0.932 (+/-0.053)[ab]

0.932 (+/- 0.053, $p<0.01$) when compared to Group S, and when examined in the light of the qualitative radiographic findings appears to support the evidence for considerable resorptive activity in the proximal fragment.

5.5 Summary

Following osteotomy,

(i) the standard group (Group S) showed a progressive increase in weightbearing with time. Axial load, displacement and strain were greatest at fourteen days, and then declined in a linear fashion to low values by six weeks.

(ii) the rigid group (Group R) exerted significantly higher GRF than the other groups in the early phase of healing. Consequently axial strains were only 25% less than those of the standard group at fourteen days, and these converged in the late phase of the experiment.

(iii) the periosteally devascularised group (Group D) displayed progressive limping and persistently high axial strains, consistent with a deficiency of structurally-competent tissue between the fragment ends.

6. ESTIMATION OF BONE BLOOD FLOW AND MINERAL UPTAKE

6.1 Introduction

In view of the important role ascribed to blood flow in the maintenance and repair of bone, the search for a simple, accurate and reliable technique of haemodynamic measurement has been intense, not only in relation to fracture healing but also for the assessment of bone pathology and the response to surgical treatment such as bone grafting.

6.1.1 Historical review

The complex organisation of the capillary systems in and around the diaphysis of long bones has hampered attempts to isolate the many afferent and efferent arms of the circulatory network in bone. Hence direct measurement of bone blood flow is difficult; most of the early work and a great proportion of recent studies have involved the use of systemically or locally injected radionuclides which are either taken up by the bone crystal or matrix or lodge in capillaries in bone (Tothill, 1984). Flow may then be determined from radioactivity detected either by external counters or by harvesting of bone samples for post mortem counting.

The Fick principle, which states that the amount of a substance accumulating in an organ (the extraction) is the difference between the inflow and outflow of that substance, is the basis for the measurement of clearance of 'bone-seeking' tracers from the circulation. Clearance is defined as the product of the blood flow and the extraction, and has been estimated using analogues of calcium such as Strontium-85 (Copp and Shim 1965), Calcium-45 (Bosch 1969) or other minerals such as Potassium-42 and Rubidium-86 (Kane and Grim 1969). However this technique assumes either complete or constant rate extraction for calculation

of flow, and it has been shown that at high flows, extraction of diffusible tracers is not uniform and may be subject to 'diffusion limitation' (McCarthy and Hughes 1984). These effects would probably be magnified in the developing fracture callus.

Washout of diffusible radioactive tracers such as iodo-antipyrine from the tissue of interest has also been used, and relies on the assumption that the rate of removal is proportional to the blood flow reaching the tissue (Paradis and Kelly 1975, Marcus, Bischof and Heistad 1981, Tothill and Hooper 1985). Non-radioactive tracers such as inhaled hydrogen gas have also been employed (Whiteside et al 1978) but require insertion of an electrode into the bone, and together with the clearance methods may be subject to errors due to recirculation or back-diffusion of the tracer (Tothill 1984).

A more satisfactory approach was the introduction of radiolabelled particles which are trapped in the capillaries or arterioles of the organ; Kane and Grim (1969) were first to describe the use of 16-25 micron diameter glass particles labelled with Sodium-24 in bone. Lunde and Michelsen (1970) made the first systematic measurements of regional cortical and medullary flow in the rabbit using plastic microspheres of 15 microns diameter. However the microsphere method is generally limited to experimental animals, which must be killed after administration of the tracer. The introduction of newer radiolabelled particles with high extraction rates such as aggregated albumin microspheres (Tondevold and Eliasen 1982) and lipid-soluble desmethylimipramine (Little and Bassingthwaighte 1983) heralds the non-destructive use of this method to measure bone blood flow, with a potential application in humans.

Another significant development in recent years has been the advent of flow measurement based on the Doppler principle. Flow is related to the shift in frequency of an applied ultrasonic signal by the movement of particles (e.g. red blood cells) at an interface beneath the probe. While this technique has an established clinical role in the

measurement of flow in large arteries, application to the microcirculation has proved difficult. As yet, detailed measurements of regional flow are not practicable, but improved instrumentation and resolution has yielded promising results, particularly in microvascular surgery (Blair, Brown and Greene 1988).

Systems quantifying the Doppler shift with laser light have been applied to the microcirculation in bone and skin and seem to correlate with microsphere flow measurements (Swiontkowski et al 1986). The laser Doppler technique also offers the advantage of 'realtime' assessment of flow changes in small regions of bone tissue. However, laser Doppler probes measure 'flux' rather than flow, are invasive, and may be subject to movement artefacts. At present, the microsphere technique remains the standard against which newer methods will be tested.

6.1.2 Physics of gamma radiation

The emission of energy from radioactive substances in the form of ionising radiation is generally either alpha particles (positively-charged Helium nuclei), beta particles (negatively-charged electrons) or gamma photons (no net charge or mass). High-energy gamma rays are similar to x-rays in their interaction with matter and are frequently used in medicine for diagnostic purposes. The emission of energy allows the unstable radionuclide to return to a more stable state, and this process is described as decay. The rate of decay of a radionuclide follows an exponential function (Aird 1975):

$$N = N_o \, e^{-ct}$$

where N = number of atoms at time t, No = initial number of atoms, and c = decay constant. A more appropriate measure of decay is the half-life ($T_{1/2}$), or the time taken for the

nuclide to decay to half its initial activity, which is given by:

$$T_{1/2} = \frac{\log_e(2)}{c} = \frac{0.693}{c}$$

Gamma radiation may be detected and measured by the interaction of photons with crystals such as sodium iodide, which produce a flash of light. These flashes produce an electron pulse when incident on a photo cathode in a scintillation counter, which is then amplified using a photomultiplier tube to give voltages which may be electronically counted. The magnitude of the pulse is proportional to the energy of the photon. The technique can therefore be used to distinguish photons of different energy arising from different radionuclides. For in vivo counting an external detector is used, and collimated to improve resolution despite a reduction in overall sensitivity. For in vitro counting of tissue samples, a crystal in the form of a well surrounding the sample improves counting efficiency.

6.1.3 Principles of the microsphere method

Essentially, the technique relies on the assumption that injection of microspheres into the arterial circulation will result in distribution of the particles in proportion to the fractional cardiac output to each organ or tissue (Gross, Marcus and Heistad 1981). The activity in each sample of tissue is compared to the activity in a reference sample of blood which is withdrawn from a peripheral artery at a known flow rate; hence, flow in the tissue sample may be calculated from this relationship. Flow measurement is therefore instantaneous and continuous monitoring is not possible, although repeated injections using microspheres labelled with different radionuclides enable haemodynamic changes to be assessed at finite time intervals.

6.1.4 Calculation of blood flow and cardiac output

Regional blood flow was calculated from the ratio of reference to sample activity, which may be arranged to give the formula:

$$Flow\ (ml/min/100g) = \frac{tissue\ activity\ x\ withdrawal\ rate\ x\ 100}{reference\ activity\ x\ tissue\ mass}$$

Using a similar formula, total cardiac output may be determined. After the terminal procedure, the syringe which had contained the microspheres was counted for residual activity along with the standard and the injection catheter, allowing estimation of the total injected dose less that retained in the injection apparatus. The corrected injected activity and whole animal mass were substituted for tissue activity and mass, respectively, in the above equation to give:

$$Cardiac\ output\ (ml/min/kg) = \frac{injected\ activity\ x\ withdrawal\ rate}{reference\ activity\ x\ animal\ mass}$$

6.1.5 Factors affecting the validity of flow measurements

Several important criteria governing the interpretation of flow measurements have been established (Buckberg et al 1971, Gross et al 1981). These are:

(i) the microspheres should be adequately mixed with blood, so that streaming in large arteries does not occur,

(ii) they should distribute in proportion to cardiac output,

(iii) they should be extracted in a single passage through the bone by trapping in small vessels,

(iv) they should not of themselves interfere with systemic or regional blood flow.

More specifically, Buckberg et al (1971) argued from a statistical point of view that at least 400 microspheres per reference or tissue sample would be required to give less than 10% error in calculation of flow. However because of the relatively low overall blood flow to the skeleton, estimated to be about 10% of cardiac output (Gross, Heistad and Marcus 1979, Morris and Kelly 1980), large doses of microspheres would be required for detailed measurements on small samples of bone, with consequent disadvantages in terms of cost and cardiorespiratory compromise. For practical purposes, injection into the left ventricle is technically easier than into the left atrium and probably achieves acceptable mixing. Li, Bronk and Kelly 1989 have also shown that for cortical bone, as few as 100 microspheres per sample gives results within 10% of those obtained with > 400 microspheres.

The dimensions of the particles are also significant, as nonentrapment will tend to cause underestimation of blood flow. Microspheres less than 10 microns in diameter may pass through the capillary bed via arteriovenous shunts (Archie et al 1973) and most studies have recommended the use of 15 micron particles (Marcus et al 1976), which approximate the diameter of the Haversian canal which contains the capillary and a small fluid space (Figure 6.1). Triffitt and Gregg (1990) have shown that for calculation of flow in the femoral cortex of the rabbit, 11 micron diameter microspheres were not significantly different when compared with the larger type and were less expensive, though if greater than 6 million were injected this frequently resulted in arterial hypotension.

The use of microspheres for measurements of regional flow and cardiac output in the adult sheep has been validated by Hales (1973), who injected 15 million spheres of 15 +/- 5

microns diameter into the left ventricle, with a reference catheter in the femoral artery. Although bone was not examined, flow in resting skeletal muscle was 5 +/- 0.8 ml/min/100g and cardiac output was 3.47 +/- 0.28 litres/min, with a mean arterial pressure of 103 +/- 3 mmHg and heart rate of 97 +/- 6 beats/min (n=13).

Typical values for bone and marrow blood flow have mainly been studied in the rabbit and dog. In the mature conscious dog, Morris and Kelly (1980) found that about 4% of the injected dose was trapped in the lung; flow in cortical bone was 2.46 +/- 0.4 ml/min/100g, cancellous bone 38.3 +/- 9.3 ml/min/100g, and marrow 7.5 +/- 4.5 ml/min/100g. Differences in flow in different parts of the diaphysis were attributed to the proportion of cortical to cancellous bone. However in a detailed study, Schnitzer et al (1982) demonstrated a gradient of flow in normal cortex, with the lowest values in the middle of the shaft of the femur and tibia, which was almost avascular. This work has been confirmed by Tothill et al (1990) and Willans and McCarthy (1991).

6.1.6 Studies of Technetium-99m MDP uptake in bone

Phosphate compounds labelled pure gamma-emitting radioisotope Technetium-99m (half-life = 6 hours) were first used for imaging of the skeleton by Subramanian et al (1972) in the rabbit. They found that following intravenous injection the complex was rapidly concentrated in bone, had no toxic effect on bone formation at low doses and was rapidly metabolised and excreted in the urine. Diphosphonates have been shown to bind directly to the hydroxyapatite crystal (Jung, Bisaz and Fleisch 1973) and to give higher bone to soft tissue ratios than other phosphate compounds such as pyrophosphate (Hughes, Jeyasingh and Lavender 1975).

From early on it was realised that although blood flow was a major determinant of the

Figure 6.1: Microsphere entrapped in capillary in cortical bone

localisation of Tc-99m MDP, the presence of immature new bone significantly affected uptake (Galasko 1975) so that at high flows the extraction measured at three hours after injection appeared to change (Siegel et al 1976). Castronovo et al (1977) showed that after injection 80% of the dose was cleared from the vascular compartment within five minutes, and that in bone there was a rapid peak followed by a steady rise in activity up to one hour. Hughes et al (1977) demonstrated that Tc-99m MDP reached bone by the process of passive free diffusion through the capillary wall, but that activity in normal bone remained constant.

In order to investigate the potential of Tc-99m MDP as a marker of bone blood flow, it was necessary to measure uptake in abnormal bone. Hughes et al (1978) reported a threefold increase in late uptake at four hours, at ten days after a canine tibial osteotomy and concluded that capillary recruitment was an important factor in explaining the increase in uptake of the radiopharmaceutical. However, in a subsequent study, uptake far exceeded the rise in flow as measured with microspheres suggesting that bone turnover was also important (Lavender, Khan and Hughes 1979).

Theoretical analysis of the washout curves of Tc-99m MDP suggested four compartments, i.e. vascular, perivascular, bone fluid and bone crystal (Hughes 1980). From studies at 1-3 hours after injection, Riggs et al (1984) argued that the non-linearity of uptake reflected diffusion-limitation: that the major control was not the delivery of the agent by the circulation, but the mechanism of concentration onto bone surfaces.

More recently the early uptake phase, in the first few minutes after administration, has been studied and it appears that at the site of bone injury, uptake in the first 60 seconds is directly proportional to blood flow (Nutton, Fitzgerald and Kelly 1985). At this stage much tracer is also present in the soft tissues and therefore accurate external counting may be limited to subcutaneous sites. Clinical investigation of the blood-pool

phase (Deutsch, Gandsman and Sparagen 1981) has yielded encouraging results in the prediction of healing of tibial fractures (Jacobs et al 1981) although the quantification and timing of the scan is controversial. Immediately after fracture Gregg et al (1986) found that the results were equivocal, although a useful relationship between early phase uptake and time to union has been obtained at two weeks (Smith et al 1987) and six weeks (Auchincloss and Watt 1982, Oni et al 1989).

The exact mechanism for the uptake of Tc-99m MDP after a fracture remains unclear and therefore interpretation of quantitative imaging is limited. Autoradiographic studies have demonstrated that the isotope is eventually found at the mineralisation front between existing bone and osteoid, and at the borders of osteocyte lacunae (Einhorn et al 1986). False-positive images of Tc-99m MDP localisation on the surface of devascularised bone grafts have also been reported (Shaffer et al 1987), but whether this diminishes the clinical potential of bone scanning is not yet certain.

6.2 Technique of microsphere administration

6.2.1 Calculation of required dose

Microspheres of 15.5 +/- 0.1 micron diameter (New England Nuclear Co, Southampton, England) were chosen for use in this study. These particles, made of a plastic copolymer, were obtained commercially labelled with Cobalt-57 which has a half-life of approximately 271 days and a principal photon energy of 122-136 keV.

The number of counts per sphere were determined by smearing a small aliquot of microspheres (0.1 ml) onto a piece of graph paper. The absolute number of spheres were then counted in a systematic manner under low power in the light microscope, and there was

no evidence of aggregation. The graph paper was then placed in a 10 ml plastic tube and counted for 300 seconds in a computerised automatic gamma scintillation counter (LKB-Wallac 1282 Compugamma, Wallac Oy, Turku, Finland) which contained a 3 x 3 inch well-type sodium iodide crystal.

In the animals used in the preliminary studies, approximately 4.5 million microspheres were injected into the left ventricle, and post mortem specimens of cortical bone weighing 1-2 g from the region of the osteotomy site were counted along with the graph paper. This revealed that an activity of 5000-7000 counts per minute per sample, equivalent to 100-150 microspheres, were obtainable with this dose. While this was not as high as theoretically required, the large number of animals in the study, the prohibitive cost of the spheres and the risk of haemodynamic effects from larger doses resulted in a compromise which was felt to be acceptable, and this dose was used consistently throughout the study.

6.2.2 Preparation of microspheres

The microspheres were supplied as a 20 ml suspension in 0.9% saline with 0.01% Tween-80, a surfactant to prevent aggregation. Prior to injection, approximately 3 ml were drawn into a 5 ml plastic syringe, the barrel and plunger of which had been previously coated with a silicone solution (Sigmacote, Sigma Chemical Co, St Louis, Missouri, USA) to prevent adhesion of the spheres. The activity of the syringe was counted for 30 seconds at the same time as another syringe containing about 0.25 ml microspheres, which acted as a standard for each injection. The activity was recorded and the microspheres were then sealed in a lead pot prior to injection.

6.2.3 Terminal operative procedure

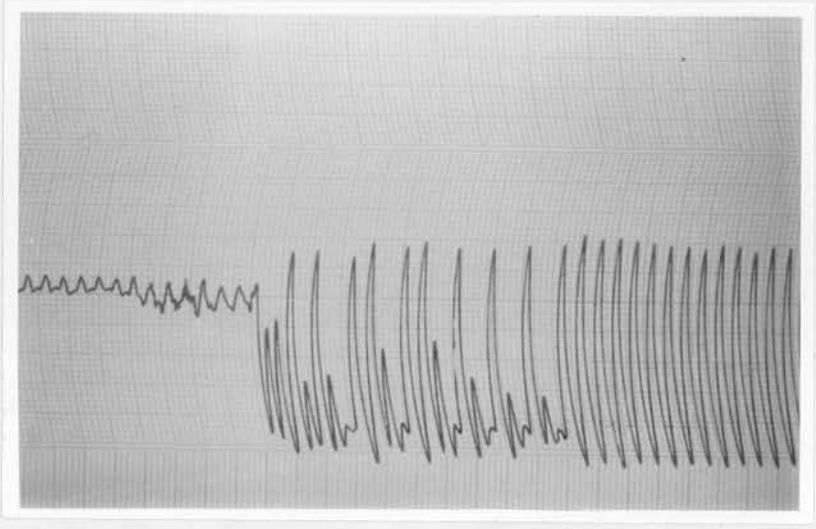
At the appropriate stage for each group, the animals were anaesthetised and intubated as described in Section 4.3.2, and uptake measurements of Tc-99m MDP performed, as will be described in Section 6.4.2. The animals were positioned supine on the operating table and a midline incision made in the neck. The right common carotid artery was exposed through the carotid sheath with protection of the vagus nerve. The artery was then ligated proximally and a short 12 gauge cannula inserted and tied into the distal lumen.

Through this a 6 French gauge Hilal aortographic catheter, measuring 65 cm in length, was passed (William Cook Europe ApS, Bjaeverskov, Denmark). The catheter had an end opening and several side holes at the tip, was filled with 0.9% saline and attached to a pressure transducer and chart recorder (Harvard Apparatus Ltd, Edenbridge, UK). A graphical recording of the arterial pressure wave was obtained as the catheter was advanced into the common carotid trunk, brachiocephalic trunk and aortic arch in turn. The tip was located in the left ventricle, as confirmed by a sudden change in the recorded pressure pulse. Details of aortic and ventricular pressures and heart rate were noted (Figure 6.2).

The right forelimb was then prepared and a short incision made just proximal to the radiocarpal joint. The radial artery was isolated and ligated distally, and a 3 French gauge intravenous cannula inserted and passed retrogradely into the brachial artery. It was then secured and attached to a fixed rate withdrawal pump (Harvard Apparatus Ltd) set to run at 0.852 ml/min. Patency of the catheter was confirmed by commencing withdrawal of arterial blood 30 seconds prior to injection of the microspheres.

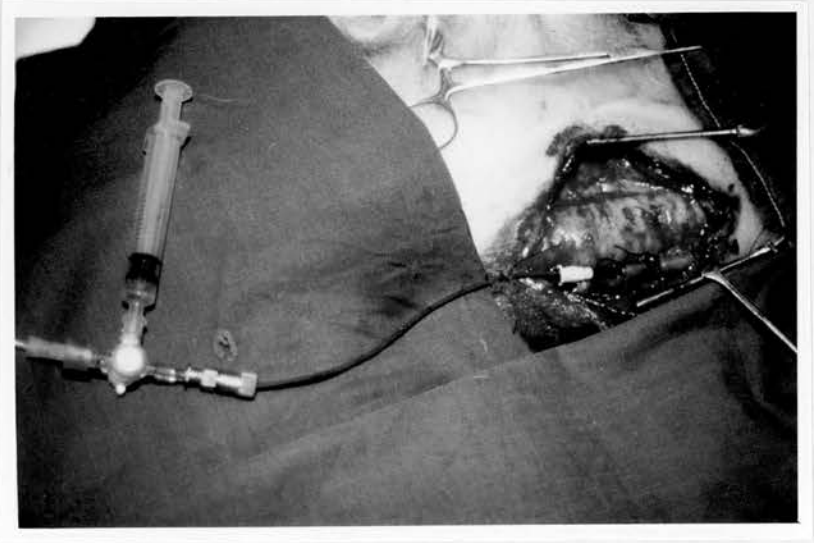
The microspheres were agitated to ensure adequate suspension and injected over 30 seconds into the left ventricle (Figure 6.3). Withdrawal of blood from the peripheral catheter continued for a further 1.5 minutes, after which an intraventricular injection of 20 ml

Figure 6.2: Recording of pressure wave in ascending aorta



aorta valve ventricle

Figure 6.3: Injection of microspheres into left ventricle



saturated potassium chloride resulted in an immediate cardiac arrest which was confirmed by the pressure recorder.

6.2.4 Preparation of tissue samples

After several minutes the hindlimbs were amputated through the knee joints and the soft tissues dissected from the bones. The crural musculature, tibiae and metatarsal bones were kept, and a segment of lowermost rib taken. After torsional testing of the tibiae the bones were frozen at -20 degrees Celsius and then thawed when convenient for further preparation. The relatively long half-life of Co-57 meant that this could be delayed for 1-2 months without significant loss of activity.

The diaphyses of both left and right tibiae were divided transversely into 15 segments about of one centimetre length using a bandsaw, and then further divided into cortical and medullary fractions with a curette. This provided samples of between 0.5 and 2 g which were placed individually into 10 ml plastic tubes. In addition, four samples from the cranial and caudal muscle compartments at the level of the osteotomy and an equivalent region of the contralateral limb were taken, together with four segments of the left and right metatarsal diaphyses which were also divided into cortex and marrow. These samples were taken in order to assess haemodynamic changes in adjacent soft tissue and in other bones of the limb.

6.2.5 Counting procedure

Each tube containing the tissue samples was weighed on an electronic balance (Sartorius Gmbh, Gottingen, Germany) and the tissue weight calculated by subtracting the weight of

the tube. All the samples from each sheep were counted with the reference blood sample in the automated gamma counter for 300 seconds per sample. Windows were set at 115-135 keV to coincide with the energy peak of the Co-57 isotope. The individual counts were stored on floppy disk and printed out at the conclusion of the counting procedure.

6.3 Regional haemodynamic measurements

6.3.1 Normal tibia

The calculated dose of microspheres administered to the osteotomised animals in the preliminary studies resulted in adequate numbers of spheres per sample for statistical purposes; however it soon became apparent that the samples from the normal sheep contained much fewer spheres, and the consequent greater error for each animal was offset by increasing the number of normal animals measured to six, rather than three animals, as originally intended. In the right tibia, mean cortical flow was 0.38 (+/- 0.14) ml/min/100g and mean medullary flow was 0.57 (+/- 0.24) ml/min/100g.

Blood flows to the whole extent of the diaphysis were determined by calculating the mass of all the cortical or medullary samples, pooling the counts (corrected for background activity) and applying the values to the flow equation as if the activity represented a single, large sample. This therefore included and was biased by the sections adjacent to the osteotomy itself, but was thought to reflect flow on an 'organ' level.

Figure 6.4 shows the distribution of flow in the normal tibial diaphysis from proximal to distal. In the cortex, there was evidence of relative avascularity, with very low calculated flows (< 0.20 ml/min/100g) at about 60% along the shaft, close to where the osteotomies were performed in the main experiment. It was interesting to note that at the

same level, the highest medullary flows were recorded (1-2 ml/min/100g), although medullary flow was generally higher than cortical flow throughout the bone. This corticomedullary discrepancy may reflect either capillary trapping of microspheres at the anastomosis between the descending nutrient arterial branches and the ascending branches of the distal metaphyseal network, or alternatively a variation in the spatial arrangement of capillary networks. While it is generally regarded that medullary and cortical vessels lie in parallel (Lopez-Curto, Bassingthwaighte and Kelly 1980), the finding of low cortical flows raises the possibility of precortical trapping in medullary vessels in series with the cortical vessels.

There was reasonably close agreement between the cortical flows for equivalent levels in right and left tibiae, although this did not hold as well for the medulla. Figure 6.5 shows the correlation for mean cortical flows which was high ($r= 0.82$, $p<0.01$). The correlation for medullary flow was lower and did not reach statistical significance ($r= 0.45$, $p<0.08$).

6.3.2 Tibial diaphysis: operated groups

Application of the external fixator alone (Group F) was associated with a moderately large but significant rise in mean diaphyseal flow compared to the normal group (Group N). Mean cortical flow was increased over six times to 2.62 (+/- 1.21) ml/min/100g ($p<0.01$) while medullary flow rose more than tenfold to 9.95 (+/- 6.15) ml/min/100g ($p<0.01$). Table 6.1 demonstrates that the cortical increases in Group F were mainly found, as expected, in the regions of pin placement; a similar pattern but of greater magnitude was found with medullary flow. However even in the section of bone between the groups of pins cortical flow was increased compared to Group N ($p<0.05$).

Figure 6.4: Distribution of blood flow in normal tibia

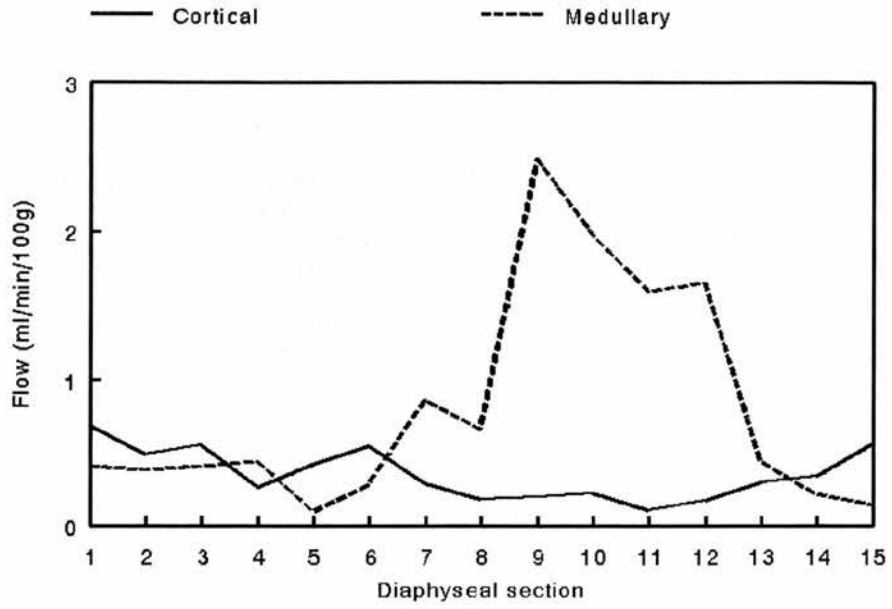
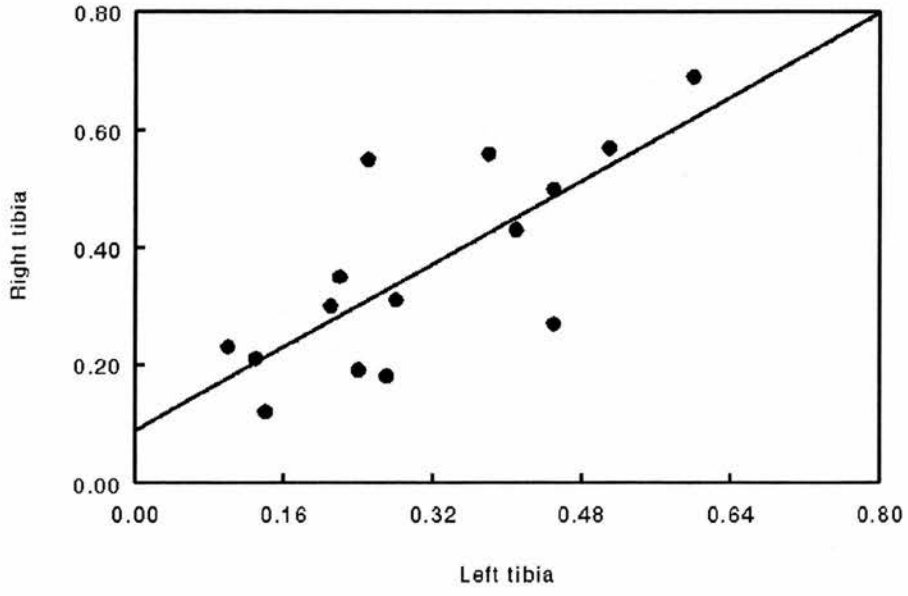


Figure 6.5: Right : Left correlation of normal blood flow



In the main experimental groups, the highest values for diaphyseal flow were found at two weeks after osteotomy. Analysis of variance showed no significant differences in cortical flow, although mean flow in the rigid group (Group R) was less than half the flows in Groups S and D, which showed rather large standard deviations from the mean (Table 6.2). In the early phase of healing, medullary diaphyseal flow in Group R was significantly lower ($p<0.01$) than in Group S. At six weeks there was a persistent rise in diaphyseal marrow flow in the devascularised group ($p<0.001$), but although flows remained well above those in the normal group, there were no other major differences between the main groups.

6.3.3 Osteotomy region

It became obvious that these differences at the 'organ' level in the diaphysis were considerably influenced by the haemodynamic events occurring at the osteotomy site. The destructive nature of the post-mortem mechanical tests meant that it was not possible to study blood flow in the actual gap tissue, which was also of small quantity. Therefore interest was confined to the sections one centimetre proximal and distal from the osteotomy gap, and a figure for cortical and medullary flow to the osteotomy region was calculated from the pooled weights and counts for these two sections, in each animal.

Analysis of variance showed significant differences in cortical ($F= 13.87$, $df= 2,14$ $p<0.001$) and medullary ($F= 5.29$ $df= 2,14$ $p<0.05$) flow in the region of the osteotomy at two weeks but only in medullary flow at six weeks ($F= 7.27$ $df=2,14$ $p<0.01$). Blood flow in periosteal callus was measured separately at 42 days in the well-vascularised groups and was not significantly different; in Group S it averaged 13.88 (+/- 7.67) ml/min/100g and in Group R it averaged 21.40 (+/- 14.66) ml/min/100g.

Further analysis of the well-vascularised groups using the t-test demonstrated that at two

Table 6.1: Effect of fixator application on normal diaphyseal blood flow

Cortical section	Normal	Fixator
1	0.69 (+/-0.50)	4.10 (+/-3.00)
2	0.50 (+/-0.17)	1.88 (+/-0.74)
3	0.56 (+/-0.29)	2.69 (+/-0.99)
4	0.27 (+/-0.24)	2.17 (+/-1.71)
5	0.43 (+/-0.34)	3.32 (+/-1.69)
6	0.55 (+/-0.51)	3.29 (+/-2.52)
7	0.30 (+/-0.17)	1.95 (+/-1.81)
8	0.19 (+/-0.08)	0.62 (+/-0.44)
9	0.21 (+/-0.11)	0.50 (+/-0.47)
10	0.23 (+/-0.11)	0.50 (+/-0.14)
11	0.12 (+/-0.18)	1.64 (+/-1.63)
12	0.18 (+/-0.15)	3.12 (+/-1.37)
13	0.31 (+/-0.40)	2.97 (+/-1.60)
14	0.35 (+/-0.43)	5.89 (+/-4.07)
15	0.57 (+/-0.59)	4.90 (+/-2.73)
Mean	0.38 (+/-0.14)	2.62 (+/-1.21)

Flow expressed as ml/min/100g tissue.

Table 6.2: Mean diaphyseal blood flow

	Rigid	Standard	Devascularised
14 days:			
Cortical	3.87 (+/-0.81)	8.55 (+/-4.92)	8.40 (+/-5.30)
Medullary	12.14 (+/-3.72)[a]	29.20 (+/-10.36)[a]	21.23 (+/-8.70)
42 days:			
Cortical	6.33 (+/-4.41)	3.11 (+/-1.53)[b]	8.68 (+/-3.80)[b]
Medullary	11.74 (+/-12.27)	5.96 (+/-2.22)[c]	22.96 (+/-8.84)[c]

Flow expressed as ml/min/100g tissue.

weeks after osteotomy in Group S, both cortical (19.54 +/- 10.59 ml/min/100g) and medullary (51.74 +/- 14.22 ml/min/100g) blood flow were about four times higher than in Group R ($p<0.01$), which recorded flows about five times higher than those of the normal group (Figure 6.6). By six weeks flows in both groups had returned toward but were still much greater than normal flows, and approximated 2-5 ml/min/100g in the cortex and 15-25 ml/min/100g in the medulla, with no statistical difference (Figure 6.7).

Comparison of the devascularised group with Group S revealed that although medullary blood flow was similar (31.20 +/- 21.47 ml/min/100g), there was a marked reduction of cortical flow measured at two weeks (1.70 +/- 1.65 ml/min/100g, $p<0.01$) as a consequence of the periosteal insult (Figure 6.6). By six weeks, as the cortical flow in Group S was falling toward baseline values, the low flows in Group D persisted so that there was no statistical difference (Figure 6.7). However the most striking feature of this group was the massive rise in medullary flow which was more than 75 ml/min/100g, or about thirty times that of the normal group, at the conclusion of the experiment.

6.3.4 Relationship to axial gap strain

The fact that at fourteen days, the standard group demonstrated a fourfold greater blood flow with only 25% greater axial strain than the rigid group, but that at 42 days there was no difference between groups in either parameter, suggested that there might be a relationship between the mechanical environment and the vascular response. Plotting the raw data for cortical blood flow versus axial strain for both two and six week experiments confirmed that at low strains the flow-strain relationship was roughly linear, but that for strains above about 50%, small increases in strain were associated with large flow responses. This relationship best fitted a logarithmic curve, and therefore the values of the dependent variable (blood flow) were transformed to logarithms and analysed using

Figure 6.6: Osteotomy regional blood flow at 14 days

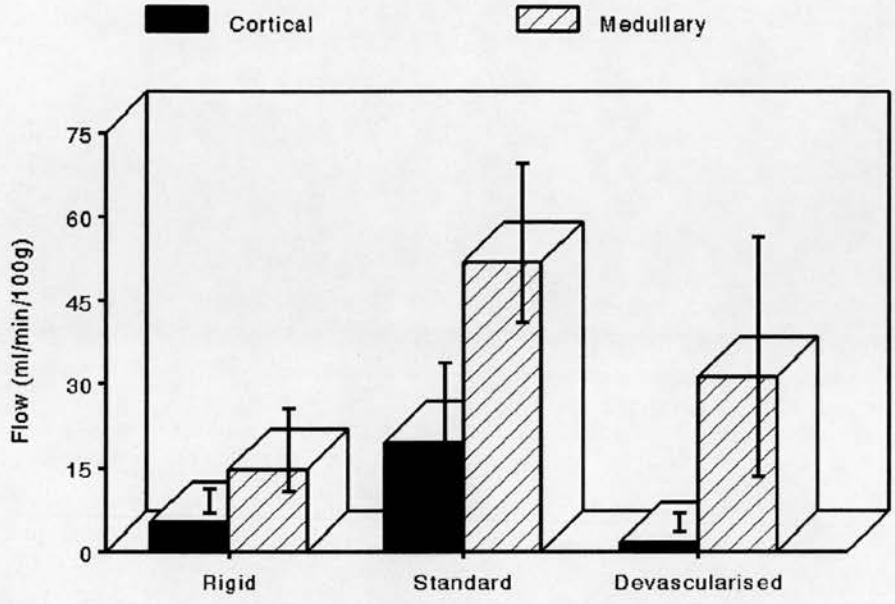
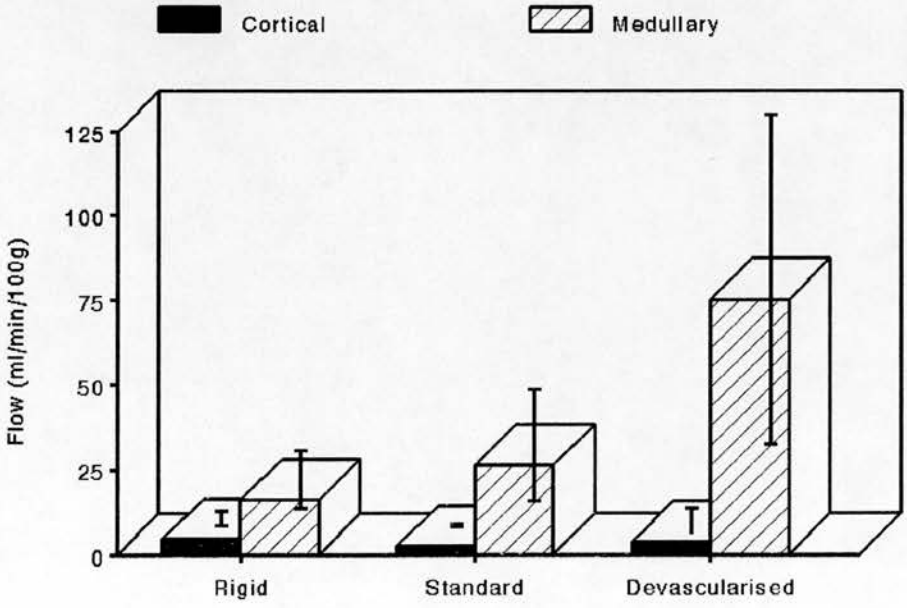


Figure 6.7: Osteotomy regional blood flow at 42 days



simple linear regression (Figure 6.8).

This revealed a significant relationship for both cortical ($r = 0.73$, $p<0.001$) and medullary flow ($r=0.50$, $p<0.05$); however interpretation of this finding is limited by the fact that flow data were only obtained at two instances in time.

6.3.5 Muscle and metatarsal

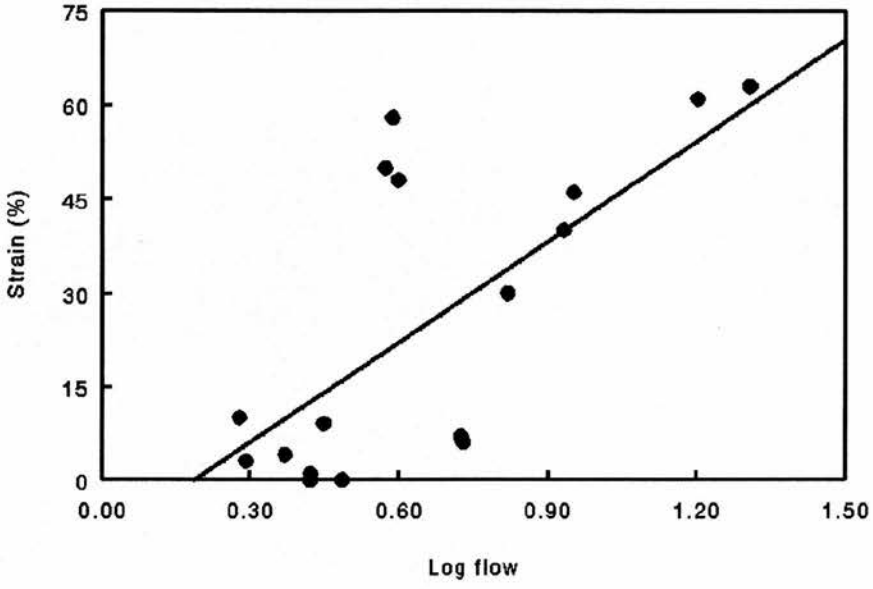
There were no differences between groups in relation to blood flow in the anterior and posterior muscle compartments at the level of the osteotomy. Typically values were from 2-3 ml/min/100g, which were similar to those found in normal muscle (Group N) and in agreement with other studies (Marcus, Bischof and Heistad 1981).

Counts obtained in the metatarsal samples were generally low and frequently only background counts were recorded. The low numbers of microspheres trapped in the cortex and marrow meant that flow calculations were unreliable, but it indicated that much of the metatarsal diaphysis was not being perfused in the anaesthetised animal.

6.3.6 Cardiac output

Mean cardiac output in the normal group was 63 ml/min/kg bodyweight, and there were no significant differences with or between the experimental groups either at two weeks (F= 57, R= 60, S= 63, D= 89 ml/min/kg) or at six weeks (R= 55, S=79, D= 58 ml/min/kg). These results confirmed that the measured haemodynamic responses at the osteotomy site were not due to generalised alterations in the systemic circulation.

Figure 6.8: Relationship of axial gap strain to cortical blood flow



6.4 Measurement of Technetium-99m methylene diphosphonate uptake

6.4.1 Radionuclide dose

The activity of the Tc-99m MDP used in this experiment at the time of injection was approximately 200 MBq, equivalent to one-third of the dose used clinically for bone scanning in human patients. Prior to administration, the MDP was drawn into a 5 ml plastic syringe and counted for 30 seconds at a standard distance of one metre from a sodium iodide scintillation counter connected to a digital scaler/ratemeter (Nuclear Enterprises Technology Ltd, Reading, England). The measured activity and time were noted, and the procedure repeated with the empty syringe after the injection, allowing calculation of injected activity after correction for radioactive decay.

6.4.2 Counting technique in vivo

The uptake of Tc-99m MDP was determined at two and six weeks after osteotomy in each group, and measurements were performed before the administration of microspheres at the terminal procedure. The scintillation counter was placed adjacent to the medial subcutaneous border of the right tibia, overlying the osteotomy site, and held at a fixed distance from the fixator by a special jig (Figure 6.9). The jig incorporated a collimator made of sheet lead 2 mm thick, with a central rectangular opening measuring 15 x 10 mm, which corresponded to the osteotomy site. To avoid error due to stray counts from other parts of the sheep (such as the urinary bladder) the scintillation crystal and photomultiplier tube were also surrounded by lead sheeting, and a lead blanket placed over the sheep's abdomen and pelvis.

6.4.3 Collection and processing of counting data

The MDP was injected into the right jugular vein and the time noted. Counts were collected from the osteotomy site for 5 seconds every 10 seconds for the first five minutes, and for 55 seconds every minute thereafter for 30 minutes, the 5 second difference accounting for the printing time of the scaler/ratemeter.

The raw counting data were entered into a specially-written microcomputer programme which corrected for decay and expressed the counts as a proportion of the injected dose (corrected count rate) to allow comparison between animals and respective groups.

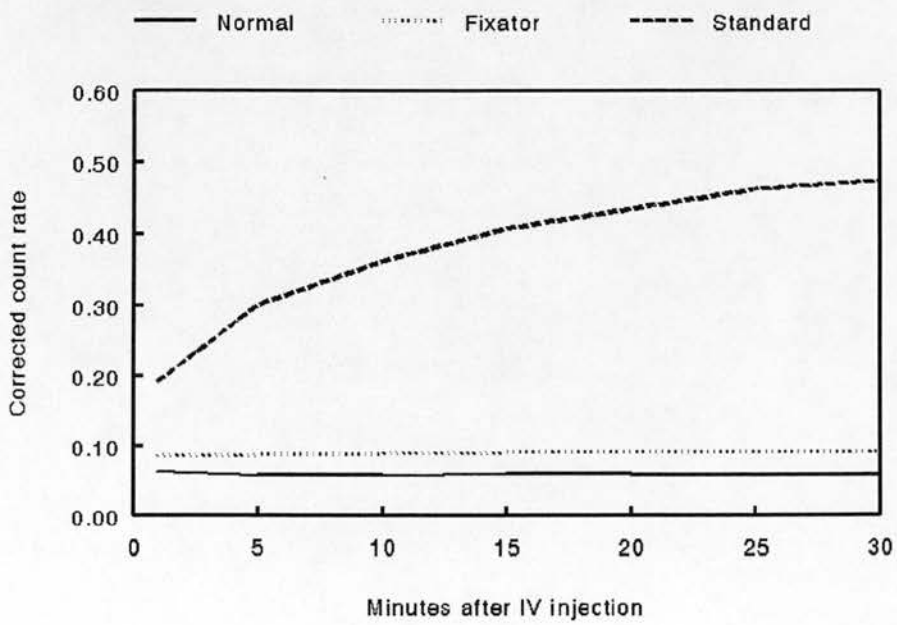
6.4.4 Analysis of results

Curves were plotted of corrected count rate against time after injection, and the means for each group tested for significant differences at each minute. Additionally, to give a 'dynamic scan value' the area under the curve (AUC) between 0-1 minute ('blood pool' phase) and between 5-15 minutes ('early uptake phase') after injection was also calculated by summing the corrected count rates (Nutton et al 1984).

Figure 6.10 demonstrates the mean uptake curves for the normal right tibia (N), the tibia to which the standard fixator had been applied for two weeks (F), and the osteotomised tibia at two weeks with the standard fixator (S). These three groups had similar values in the blood pool phase, but in the early uptake phase there were significant differences between them ($p<0.05$). The fixator-only group (F) was higher than the normal group (N) and remained so after 2 minutes, but both displayed a plateau or steady-state MDP uptake. The osteotomised tibia (S) was higher than the fixator-only group after 1 minute and instead showed a progressive increase in uptake, even at 30 minutes after injection.

Figure 6.9: External scintillation counter mounted at the osteotomy site

Figure 6.10: Effect of fixator application +/- osteotomy on Tc-99m MDP uptake



Comparison of the three main experimental groups at two weeks after osteotomy is illustrated in Figure 6.11. The well-vascularised groups (S and R) yielded very similar uptake curves which though higher in Group S were not significantly different. Periosteal devascularisation resulted in a plateau curve reminiscent of that for normal bone and was significantly lower than Group S after 12 minutes. However, there were no differences between the AUC for the three groups in the blood pool or early uptake phases.

Similar observations were made at six weeks after osteotomy (Figure 6.12) although the absolute counts were higher in all groups. The curves for the well-vascularised groups were essentially the same, and the blood pool AUC did not separate the three groups. The early uptake AUC did show a significant difference ($p<0.05$), reflecting the lower count rate in the devascularised group when compared with the standard group after 5 minutes.

6.5 Summary

(i) There was regional variation in blood flow to the marrow and cortex of the normal tibial diaphysis,

(ii) osteotomy and external fixation resulted in significant increases in general diaphyseal blood flow, particularly in the medullary cavity, but cardiac output was not affected,

(iii) in the early phase of healing of the well-vascularised osteotomies, modest differences in axial strain were associated with large differences in the vascular response at the osteotomy site,

Figure 6.11: Tc-99m MDP uptake at 14 days after osteotomy

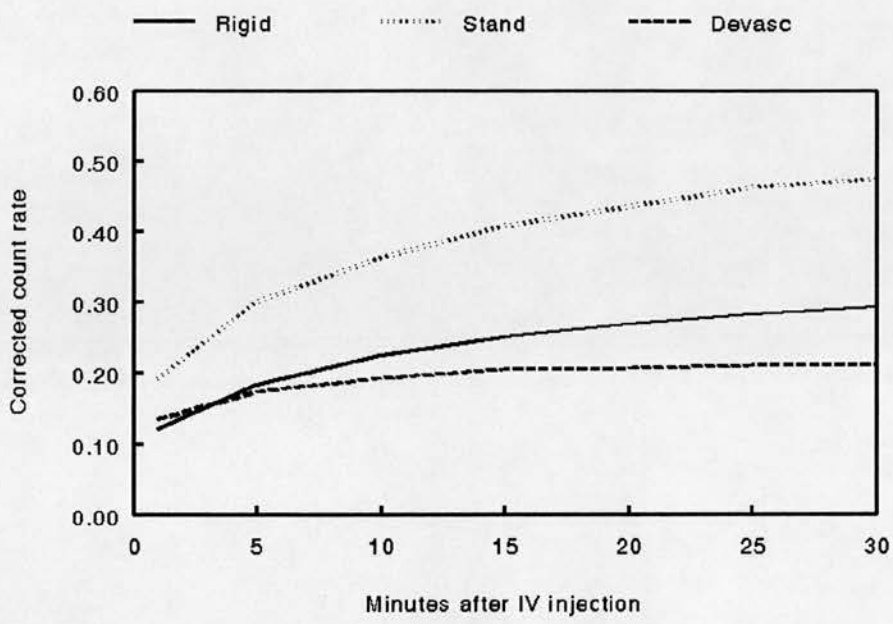
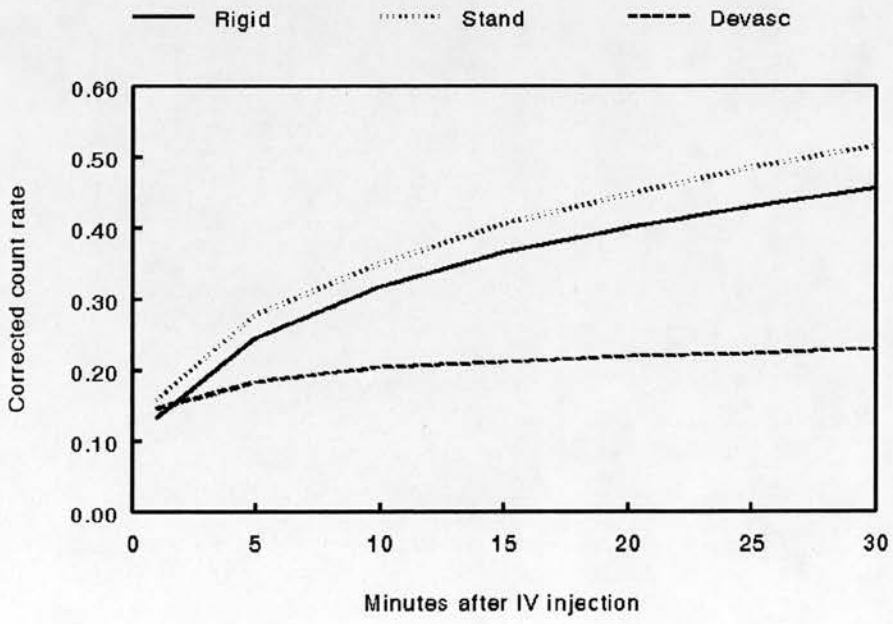


Figure 6.12: Tc-99m MDP uptake at 42 days after osteotomy



(iv) this was associated with a significant increase in mineral uptake at two and six weeks compared to the normal or non-osteotomised externally-fixed tibia, though there was no demonstrable difference between the two osteotomised groups,

(v) periosteal devascularisation resulted in persistently low cortical blood flows, significantly lower mineral uptake and evidence of endosteal resorption despite a large rise in medullary flow.

7. MECHANICAL TESTING OF HEALING BONE POST MORTEM

7.1 Introduction

7.1.1 Composition of bone

Fresh bone may be regarded as a two-phase composite material which consists of a mineral phase of bone salts embedded in a fibrous organic matrix. By weight, bone is about 20% water, 45% bone salts and 35% matrix (Carter and Spengler 1978). The structural organisation of bone is generally either cortical (or compact) with a porosity of 3-5%, or cancellous, which may have a porosity of up to 90% (Natali and Meroi 1989). When dry, human cortical bone is about 66% mineral and 33% organic; in other mammals such as cattle the mineral component is a little higher at about 76% (Quelch et al 1983). The mineral phase, which accounts for the rigidity of bone, contains large quantities of calcium and phosphate in a mixture of hydroxyapatite crystals and amorphous calcium phosphate. The organic phase is mainly Type I collagen (90%), proteoglycans (1-2%) and small amounts of other proteins such as osteocalcin (Price et al 1980) and sialoprotein (Quelch et al 1983).

7.1.2 Techniques for testing mechanical properties of bone

The basic mechanical behaviour of bone has been examined in detail in terms of its properties as a material, using small milled specimens of bone tissue, or as a structure, using whole bones or representative segments of bones.

Material testing of cadaveric samples machined to standardised dimensions has shown that cortical bone has both elastic and plastic characteristics when loaded to failure in

tension in vitro (Reilly and Burstein 1975). Plastic deformation may also occur clinically in paediatric bone (Mabrey and Fitch 1989). Moreover, bone is viscoelastic, i.e. the strain response depends both on the rate and duration of applied loading (Currey 1988), so that increasing the strain rate from 0.001 per second to 10 per second results in a doubling of strength and stiffness (Carter and Spengler 1978). Bone is also anisotropic, elongating by as much as 3% when loaded in the longitudinal axis but by less than 1% when loaded transversely (Reilly and Burstein 1975).

Clearly tests of structural properties of bone must therefore take account of these features, although variations in material properties may also arise due to intrinsic differences in microstructure, porosity, mineralisation or bone matrix. Burstein and Frankel (1971) have proposed five criteria for the structural testing of experimental bone: (i) loading should result in fractures similar to those observed clinically, (ii) the distribution of the applied load should be equal throughout the bone, (iii) the loading method must not be too dependent on bone geometry, (iv) the strain rate must be controlled, and (v) the equipment should be cheap and simple to use.

Bones have been tested in bending in either three-point or four-point configurations where the bone is supported at either end and the load is applied centrally (Henry, Freeman and Swanson 1968, Simkin and Robin 1973). However, this arrangement results in maximum stresses at the points of loading, which may not necessarily correspond to the weakest part of the specimen. The testing of bone in torsion meets all the criteria of Burstein and Frankel (1971) and although bending strength recovers more quickly after experimental fractures, torsion is generally recognised as a more 'functional' test (Ekeland, Engesaeter and Langeland 1981).

7.1.3 Effect of physical conditions

A detailed study of the effect of preparation of bone samples on mechanical properties was undertaken by Sedlin (1965) using bending tests of human femoral bone. Preservation in 10% formalin or fixation in 40% alcohol was associated with an increase in stiffness which was not reversible after immersion in Ringer's solution. Dry heat at temperatures of 100 degrees Celsius resulted in greater deformation than when bones were tested in wet heat or after room temperature drying alone, and even at 37 degrees deformation was significantly greater than at 21 degrees. However, freezing at -20 degrees for 3-4 weeks and then reheating to 37 degrees did not seem to alter the physical properties of bone.

These findings may not apply to every experiment; a recent study found a 30% decrease in torsional strength and stiffness of rat bone subjected to freeze-drying (Randall et al 1991). Concomitant high-dose gamma irradiation had a synergistic effect, whereas irradiation alone appeared to result in a significant decrease in torsional strength only after eighteen weeks in the rat, with little effect on stiffness (Maeda et al 1988).

7.1.4 Mechanical testing of fractures

The classic work in this field was performed by White, Panjabi and Southwick (1977) and their classification of the biomechanical stages of fracture repair is now universally accepted. In an investigation of externally-fixed osteotomies of the rabbit tibia, they described four characteristic curves from torque-angle plots which were associated with differences in the morphology of the fracture (Table 7.1).

In a similar model, but subject to interfragmentary compression with a bilateral external fixator, Aalto et al (1987) found recovery of torsional strength as early as three weeks

Table 7.1: Biomechanical stages of fracture healing (White et al 1977)

Stage	Pattern of failure
I	Through original fracture site with low-stiffness, rubbery pattern
II	Through original fracture site with high-stiffness, hard tissue pattern
III	Partially through original fracture site and partially through previously intact bone with a high-stiffness, hard tissue pattern
IV	Site of failure unrelated to original fracture site; high stiffness pattern

after osteotomy, reaching a maximum at six weeks despite the fact that the osteotomy line was still visible on radiographs. This is somewhat difficult to understand in the light of the findings of Panjabi et al (1985) that cortical continuity, or progressive disappearance of the osteotomy line, was correlated with torsional strength.

Recently, attempts have been made to relate variables of the fracture gap to the measurement of torsional properties. Using a complicated technique of microinterferometry, Powell et al (1989) analysed calcium content in the gap of a rat metatarsal fracture and found a sharp rise which reached a plateau at twelve weeks, while the fracture continued to regain its mechanical properties, although the author concluded that calcium content had potential in the prediction of strength. Markel, Wikenheiser and Chao (1990) confirmed these results in a canine tibial osteotomy with a 2 mm gap and found a good correlation with indentation stiffness; however, callus quantity was also found to contribute significantly to torsional stiffness, reaching a maximum at six weeks.

7.2 Torsional testing

7.2.1 Preparation of specimens

When the mineral uptake and blood flow measurements had been completed at the terminal operative procedure, the animals were killed as described in Section 6.2.3. The crura were amputated through the stifle (knee) joints and the tibiae disarticulated from the distal part of the limbs. The major muscle groups were also removed, leaving the periosteum and callus attached to the diaphysis. In Group D, the silicone rubber sleeve was incised longitudinally and removed without disturbing the medullary contents. In all the osteotomised tibiae the fixators were left attached to the bone to prevent any inadvertent handling damage to the early callus.

The bones were kept moist at about 25 degrees Celsius and tested within two hours of death. The ends of each bone were mounted in 15 mm deep stainless steel cups and centred using four small grub screws. The position was secured using Woods' metal, a rapid setting alloy which melts at 68 degrees Celsius and which hardens at room temperature without significant loss of volume. Once the bones were located, the fixators were removed and swabs taken of each pinsite for microbiological analysis (Section 4.5.4).

7.2.2 Torsional testing machine

The device (Figure 7.1) is discussed in detail elsewhere (Draper 1992) but the elements of the design were in accordance with those instruments previously published (Burstein and Frankel 1971, Jonsson and Stromberg 1985). The proximal cup was attached to a bearing driven by a constant speed electric motor; the distal cup was attached to a bearing incorporating a small beam, upon which strain gauges were mounted. When rotated the

Figure 7.1: Torsional testing apparatus

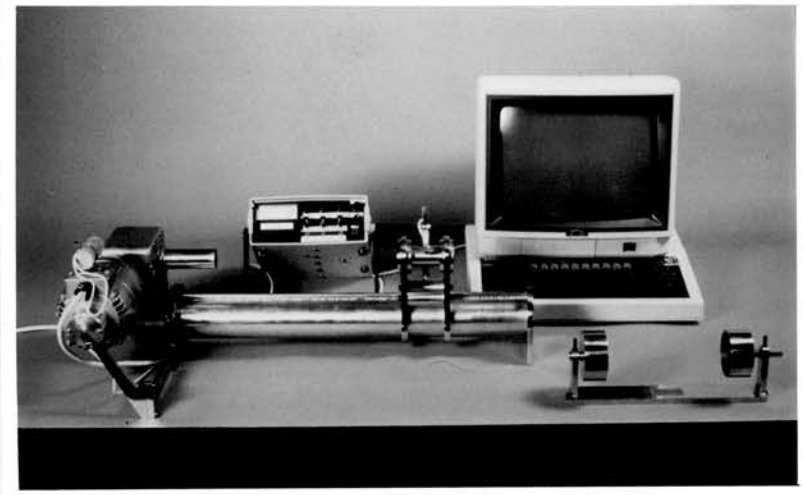


Table 7.2: Torsional properties of normal tibiae

Side	Angle to failure (degrees)	Torque (Nm)	Stiffness (Nm/degree)	Energy absorbed to failure (J)
Right	39.43 (+/-1.41)	62.12 (+/-17.34)	1.851 (+/-0.442)	19.95 (+/-5.77)
Left	43.63 (+/-4.15)	61.57 (+/-11.89)	1.785 (+/-0.460)	23.75 (+/-3.58)

deformation of the beam against a stop was calibrated to the magnitude of the torque applied by the electric motor; the angular displacement of the motor was recorded by an optical sensor. The signal from the transducer was sampled at 50 Hz by a BBC microcomputer (Acorn Computers Ltd, Cambridge, England) which calculated maximum torque, angle and energy absorbed to failure, and torsional stiffness for each bone. Since variation in length and radius may affect torsional properties, the gauge length (between cups) and minimum diameter of each tibia were measured prior to testing.

The bones were loaded to failure at a rate of 360 degrees per minute in a clockwise direction, corresponding to internal and external rotation of the right and left tibiae, respectively.

7.3 Torsional properties

7.3.1 Normal ovine tibia

Despite the opposite directions of rotation, there were no statistically significant differences between right and left tibiae in the normal sheep in terms of mechanical properties, or in length or minimum diameter. The torsional characteristics are illustrated in Table 7.2 and show good agreement between right and left sides. A typical plot of torque against angle for a normal tibia is demonstrated in Figure 7.2, which reveals the essentially triangular appearance of the curve, with only a small area of plastic deformation beyond the yield point before failure. The torsional stiffness was determined from the linear part of the curve in the elastic region, while the energy absorbed to failure represented the area under the curve up to the point of maximum torque. The typical pattern of failure in the normal bone with intact periosteum, but stripped of soft tissues, was sudden and explosive, with an extensively-comminuted long

Figure 7.2: Torque-angle plot for normal tibia tested to failure

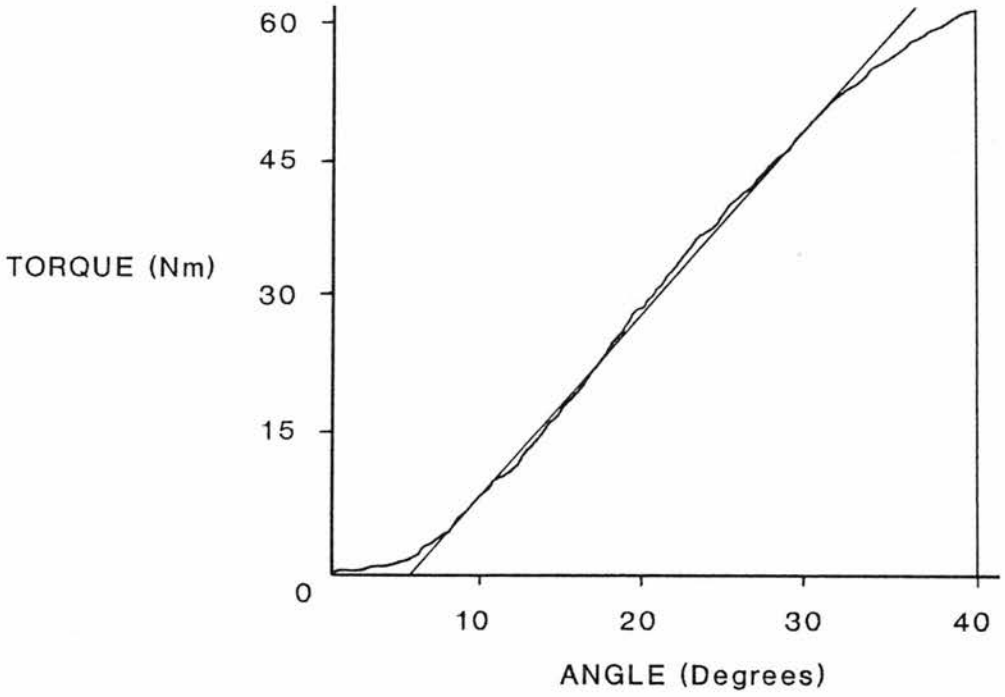
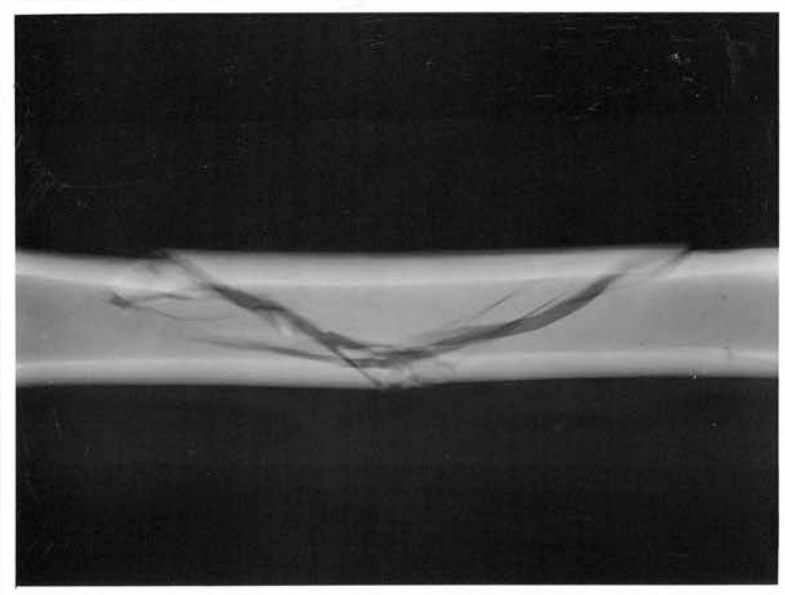


Figure 7.3: Radiographic pattern of failure in normal tibia



spiral fracture (Figure 7.3) occurring at approximately 60% of the distance from proximal to distal diaphysis.

Similar patterns of failure were observed in the intact contralateral tibiae in the main experimental groups. Analysis of variance applied to all the intact tibiae detected no significant differences between them in any parameter of the torsional tests.

7.3.2 Early phase results

The effect of application of an external fixator without an osteotomy was assessed at fourteen days. After removal of the fixator, the tibiae in Group F all failed through the most proximal hole of the distal pin group (Pin 4) and were approximately 50% weaker and 30% less stiff in torsion than the tibiae of the normal group. The angle to failure through the pinhole was about 70% of normal, but this required only about 45% of the energy required to fracture a normal bone, demonstrating the considerable local stress-raising effect of the relatively large diameter pins used in this study.

The osteotomies were classified according to Wolf et al (1977). At fourteen days all the well-vascularised osteotomies were Stage I (Table 7.3). The torsional properties of the devascularised osteotomies were so low as to be less than the resolution of the machine, and therefore no staging or quantification could be carried out.

The properties of the well-vascularised groups at fourteen days are illustrated as a proportion of the intact tibiae in each group in Figure 7.4. It is evident that at such an early stage of healing, the strength, stiffness and energy required to cause the early interfragmentary tissue to fail are equivalent to only 1-2% of those parameters in the intact bone. Using the t-test, there was no significant difference between the two well-

Table 7.3: Biomechanical staging of healing osteotomies

	ND	I	II	III
Rigid				
14 days (n=5)		5		
42 days (n=5)		2	3	
Standard				
14 days (n=4)		4		
42 days (n=5)		3	1	1
Devascularised				
14 days (n=6)	6			
42 days (n=7)	3	4		

ND = not detectable

Figure 7.4: Torsional properties at 14 days

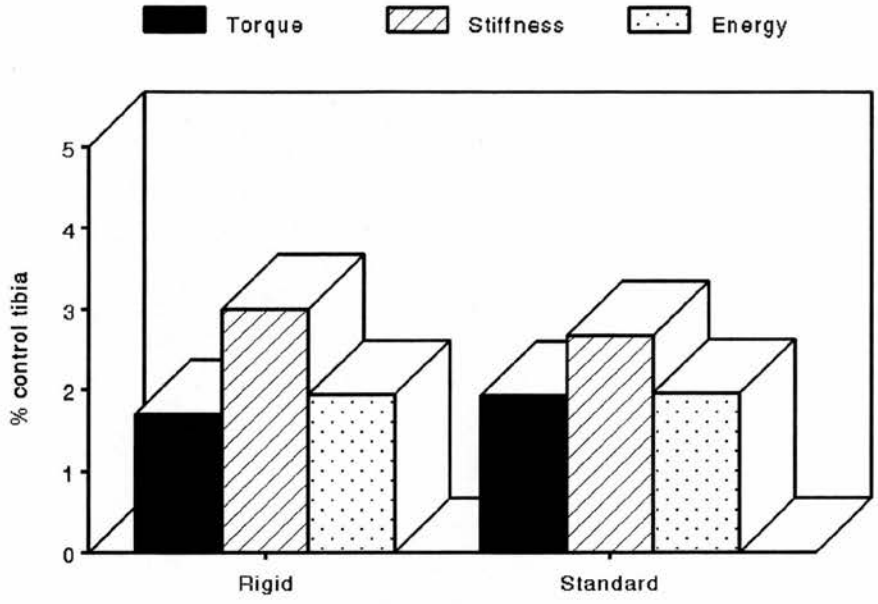
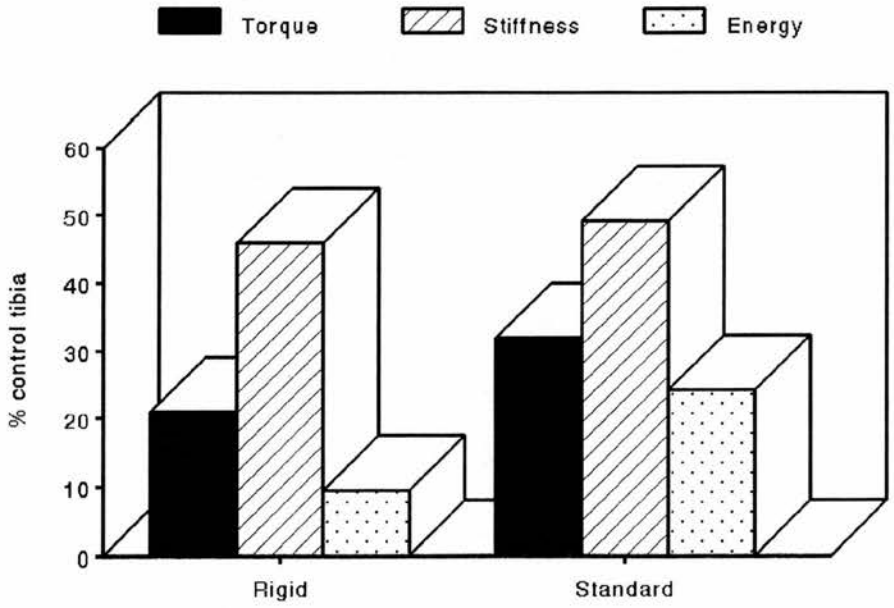


Figure 7.5: Torsional properties at 42 days



vascularised groups at this stage of healing. Surprisingly, the angle to 'failure' of this tissue was 90-95% of that for intact bone and this may have represented a small but significant volume of 'hard' tissue as a result of mineral deposition in the early callus.

7.3.3 Late phase results

By 42 days after osteotomy, there was appreciable recovery of strength and stiffness by both well-vascularised groups, (Figure 7.5) but there was little evidence of structural restoration in the devascularised group. In the rigid group, 3 of 5 osteotomies were classified as Stage II, whereas the same proportion in the standard group were classified as Stage I. In Group S, which had recorded greater axial loads, displacements and strains in the early phase after osteotomy, the values in each mechanical parameter were slightly higher than those measured in Group R, but this did not reach statistical significance. At the conclusion of the experiment the well-vascularised groups had recovered approximately 50% torsional stiffness and 25% torsional strength of the intact bones.

In Group D at 42 days, the pattern of recovery was similar to that found in the well-vascularised groups at fourteen days, indicating a substantial delay in healing ($p<0.01$). Although there was some radiographic evidence of medullary callus in these osteotomies (Figure 5.4), this did not appear to contribute to the reduction of axial displacement or strain, or to provide more than a tenuous mechanical link between the fragments.

7.4 Summary

Torsional testing of the experimental and intact tibiae was performed for each animal. Compared to a normal intact bone:

(i) application of an external fixator alone resulted in a 50% loss of strength and a 30% loss of stiffness,

(ii) at fourteen days after a well-vascularised osteotomy, and at 42 days after periosteal devascularisation, stiffness and strength were less than 2%,

(iii) qualitatively, the rigid group tended to fail with a more mature pattern, however the standard group recorded higher absolute values on testing,

(iv) there was no significant difference demonstrated between well-vascularised groups, but both had significantly greater torsional properties than the devascularised group.

8. QUANTITATIVE MICRORADIOGRAPHY AND HISTOMORPHOMETRY

8.1 Introduction

The 'benchmark' investigation in the clinical management of fractures is the standard radiograph; in experimental research the investigation of cellular activity in bone has tended to depend upon routine histological staining of thin decalcified sections of the tissue of interest. However, despite their widespread use there are several disadvantages of both these techniques which limit their value in comparative experiments.

Resolution of the spatial arrangement of mineralised tissues is often difficult with ordinary two-dimensional radiographs, even with orthogonal views. Moreover, differences in exposure between individual films means that quantitative analysis must be expressed in terms of a ratio generated for each film, e.g. density of cancellous to cortical bone. As in a routine histological section, the radiograph provides essentially a static image of the complex and coordinated process of bone formation and resorption which results in progressive remodelling of the callus to cortical bone.

The study of dynamic processes in bone has its origins with the observations of John Belchier (1736) that the bones of pigs fed on madder (Rubia tinctorum) were stained red by the dye alizarin; prompted by this Duhamel (1739) conducted a series of experiments which showed that the stain was confined to newly-forming bone on the periosteal surface (Keith 1919). A number of other compounds which undergo chelation with calcium in forming bone have been identified, of which the most widely used is the tetracycline group of broad spectrum antibiotics (Plenk 1986) which fluoresce a brilliant yellow colour when viewed under ultraviolet light.

This phenomenon led to the development of experimental techniques for the study of the rate and distribution of new bone formation. Vanderhoeft et al (1962) used pulsed doses of tetracycline in immature canine bone, and described seven stages of formation of secondary osteons (Haversian systems), from the initial resorption cavity left by the osteoclast through to the mature osteon, a process taking an average of six weeks to complete. Kelly et al (1965) used binocular fusion to compare tetracycline labelling in undecalcified sections with mineral density on high resolution 100 micron-thick microradiographs, and found that tetracycline appeared during the calcification of newly-synthesised osteoid. Labelling was noted on 10-30% of the surfaces of normal young bone but on only 2-5% of normal adult bone.

Although factors controlling the remodelling process remain poorly understood, these early studies suggested that remodelling sites were localised around sites of muscle attachment, and appeared to be more numerous in the more distal bones of the the limb (Vanderhoeft 1962) although spatial and temporal variation of remodelling within long bone diaphyses appears to be considerable (Harris 1968).

For quantitative assessment of tetracycline uptake in tissue sections many investigators have relied on the manual point-counting technique of Harris and Weinberg (1972, in Aro 1990) which is useful for studies of intracortical remodelling, but which is difficult to apply to callus because of the rapid turnover of new and previously-labelled bone. Measurement of microradiographs has proved more practicable with the advent of computerised systems which enable calculation of a range of two-dimensional parameters in cross-sections (Lloyd and Hodges 1971) and longitudinal sections (Aro, Eerola and Aho 1985b).

In this study a computerised image analysis system was used to attempt to define, with microradiography and fluorochrome labelling respectively, the spatial and temporal

relationship of new bone formation in callus and cortical bone to both the mechanical environment of the osteotomy, and the haemodynamic responses measured in each of the three experimental groups.

8.2 Experimental techniques

8.2.1 Preparation and administration of fluorochromes

Fluorescent bone-seeking markers may be administered either in a 'continuous' schedule, usually given as a daily feed supplement (Harris 1968), or as parenteral boluses given at finite time intervals (Einhorn et al 1990). Continuous labelling has the advantage that all new bone formed during the experiment will be detected and may be compared with the proportion of unlabelled bone; however, the rate of apposition at different stages cannot be determined with accuracy. In addition, there is a greater risk of interference with the process of mineralisation itself by administration of a continuous label. For these reasons a discontinuous labelling schedule was selected based upon the specific markers described by Plenk (1986), and at the doses quoted the toxic effects of an intravenous injection appear to be negligible.

The tetracycline group of antibiotics is frequently used in veterinary practice for the control of infection, and therefore sections of the lowermost right rib measuring approximately 2 cm in length were taken during the primary surgical procedure to examine for evidence of pre-administration and to assist with interpretation of the post mortem images. Oxytetracycline (yellow, maximal excitation 405-435 nm) (Terramycin, Pfizer), calcein (green, maximal excitation 495 nm) and xylenol (orange, maximal excitation 377 nm) (Sigma Chemical Company, St Louis, Missouri, USA) were chosen for their ability to give strong, reliable and contrasting fluorescence in undecalcified sections, and all

injections were made over 30 seconds into the right jugular vein of each experimental animal. Oxytetracycline (25 mg/kg) was administered immediately after the osteotomy on the day of operation. Calcein (10 mg/kg) was given at 14 days, and xylenol (60 mg/kg) was given at 28 days. The labelling procedure was performed in all animals, but a technical error in the use of an inappropriate cement for mounting the sections from the animals killed at fourteen days prevented quantitative analysis of these specimens by producing a high background fluorescence which obscured the fluorochrome labels.

8.2.2 Post mortem processing of specimens

At the time of the terminal procedure, a second segment of lowermost rib was harvested from the opposite side from which the preoperative specimen had been taken. Then, after torsional testing, the tibiae were frozen at -20 degrees Celsius until ready for sectioning. At approximately one millimetre proximal and distal to the osteotomy gap, and from the distal diaphyses of right and left tibiae, complete undecalcified transverse sections of callus and cortical bone measuring 150 microns thickness were cut using a Microslice II tensioned annular diamond edged saw (Malvern Instruments Ltd, Malvern UK) cooled with 70% alcohol. These sections were then polished by hand on ground glass prepared with carborundum (Abrasives GB Ltd, England) to achieve a uniform thickness of 120 microns, which was checked using a micrometer, and then fixed in 70% alcohol. When all sections had been cut, they were placed in contact with Kodak 4415 high resolution x-ray film and microradiographs were made by exposure in a Faxitron 43805 soft x-ray machine (Hewlett-Packard, Vinten Instruments, Weybridge, Surrey) for 5 minutes at 20kV and 1 mA tube current at 30 cm from the x-ray source. The sections were then progressively dehydrated in 70% alcohol, 90% alcohol, absolute alcohol and xylene prior to mounting in Hystomount (Histolab, Hemel Hempstead, Herts UK) for examination under ultraviolet light.

8.3 Qualitative examination of longitudinal sections

One animal from each of the three groups killed at six weeks was chosen at random and transverse sections were not taken. Instead, the whole diaphysis from 2 cm proximal to 2 cm distal to the osteotomy was prepared for longitudinal section, decalcification and conventional histochemical staining, in order to provide qualititative evidence of the pattern of tissue differentiation in various regions around the osteotomy gap.

8.3.1 Histological techniques

The bones were fixed in 10% neutral buffered formalin, and decalcified in 5% formic acid for 3-4 weeks. The endpoint of decalcification was determined using the ammonium oxalate test (Bancroft and Stevens 1990). The undecalcified specimens were then washed in distilled water and dehydrated as described above, before being placed in Histoclear clearing agent (Cellpath plc, Hemel Hempstead, Herts UK) for 48 hours until transparent. The tissue was then embedded in Paraplast tissue embedding medium (Monoject Scientific Inc, Athy, Co Kildare, Ireland), a paraffin wax compound which melts at 56 degrees Celsius. Sections measuring 7-10 microns thick were cut on a rotary microtome.

The thin sections were progressively de-waxed in xylene, absolute alcohol and 25% ammoniacal alcohol and rinsed in distilled water. The sections were first stained with alcian blue haematoxylin for 60 minutes at room temperature using the technique of Sayers, Volpin and Bentley (1987). After converting the haematoxylin pigment from red to blue in tap water, the Goldner's trichrome technique was applied (Bancroft and Stevens 1990) by staining with ponceau de xylidine, acid fuchsin and azophloxin for three minutes. Sequential rinsing in glacial acetic acid and alternate staining with orange G and phosphomolybdic acid (three minutes) and light green dye (2-5 minutes) was carried out

before final dehydration and mounting in Hystomount.

8.3.2 Microscopic appearances

The longitudinal sections were viewed under a Wild M3Z stereo light microscope (Wild-Leitz, Heerbrugg, Switzerland) at 6.5x to 40x magnification. The most striking differences were noted between the devascularised osteotomy and the two well-vascularised osteotomies.

In the devascularised osteotomy (Figure 8.1), the gap between the cortices was filled with necrotic haematoma and cell debris and there was no evidence of periosteal new bone. A feature of the underlying cortex was the prominence of empty lacunae, although scattered osteocytes were also observed. The proximal fragment was being extensively resorbed, and multinucleate osteoclasts were found in scalloped resorption lacunae all along the endosteal cortex. A cone-shaped wedge of medullary woven bone was seen to penetrate toward the osteotomy region, and was separated from the resorbing cortical surface by a highly cellular fibrous membrane. The apex of the medullary bone was almost at the level of the osteotomy gap. In the distal fragment the medullary cavity was occupied by sparsely cellular adipose tissue, with no new bone formation.

As a consequence of mechanical testing, the standard osteotomy section had failed partially through the original gap and partially through the periosteal callus (Figure 8.2). Failure within the gap had occurred at the interface between the the cortical ends of the proximal fragment and a fibrous infiltrate, although there was also woven bone present within the gap. Periosteal callus was more extensive in the standard section than in the rigid section, and there appeared to be more fibrous tissue present in both the intercortical gap and the medullary callus. The rigid osteotomy failed with a similar

Figure 8.1: Longitudinal section through devascularised osteotomy at 42 days

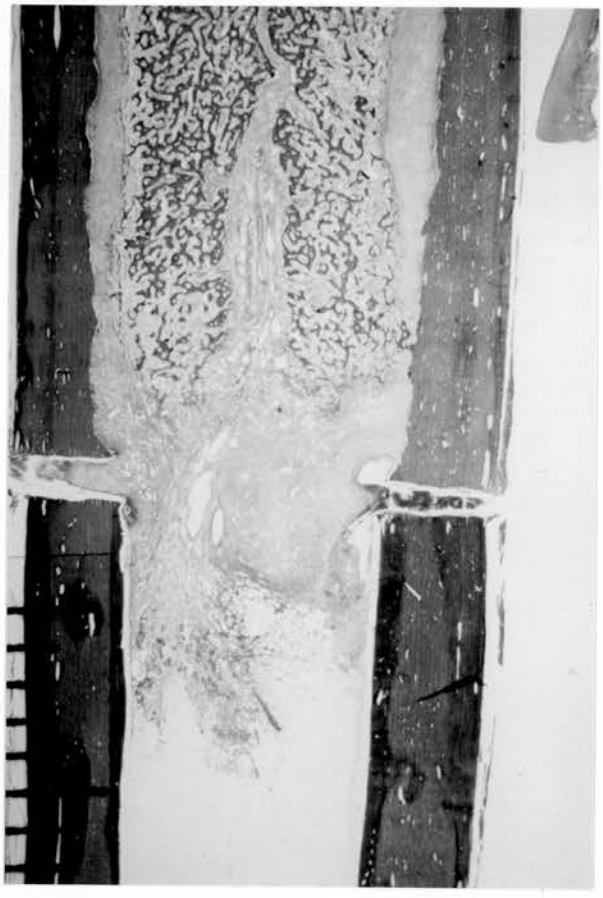


Figure 8.2: Longitudinal section through standard osteotomy at 42 days

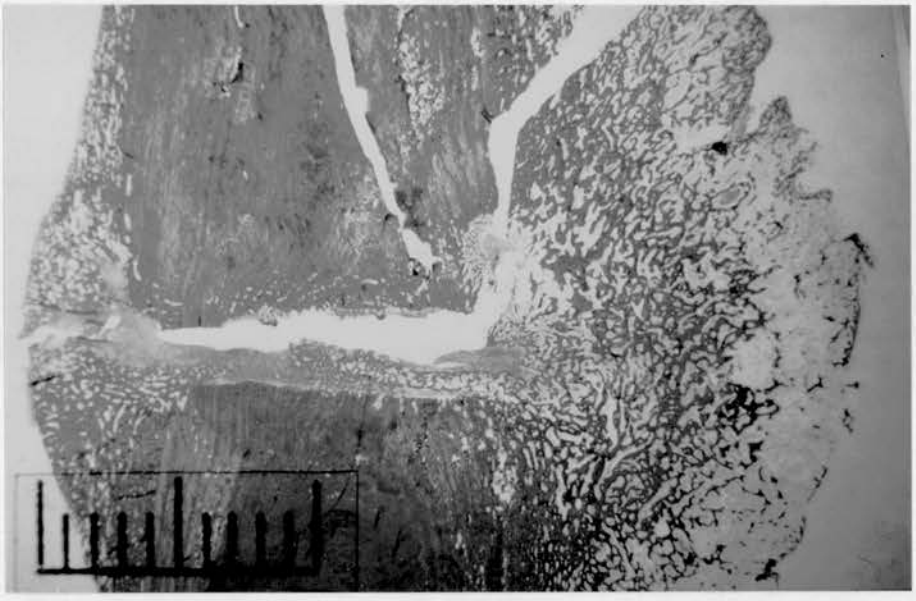
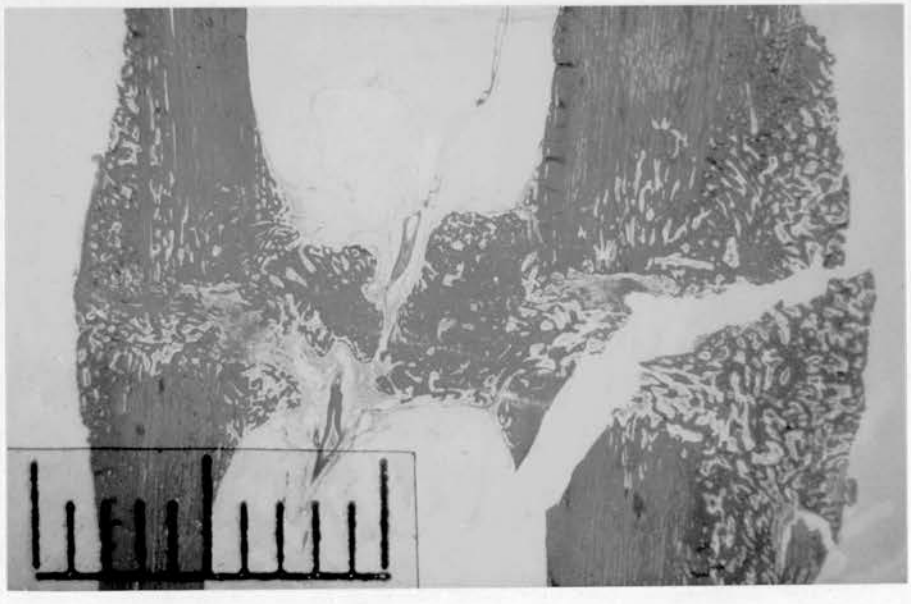


Figure 8.3: Longitudinal section through rigid osteotomy at 42 days



pattern (Figure 8.3), and as in the standard osteotomy, little cartilage was observed at this stage in the healing process.

8.4 Quantitative cross-sectional image analysis

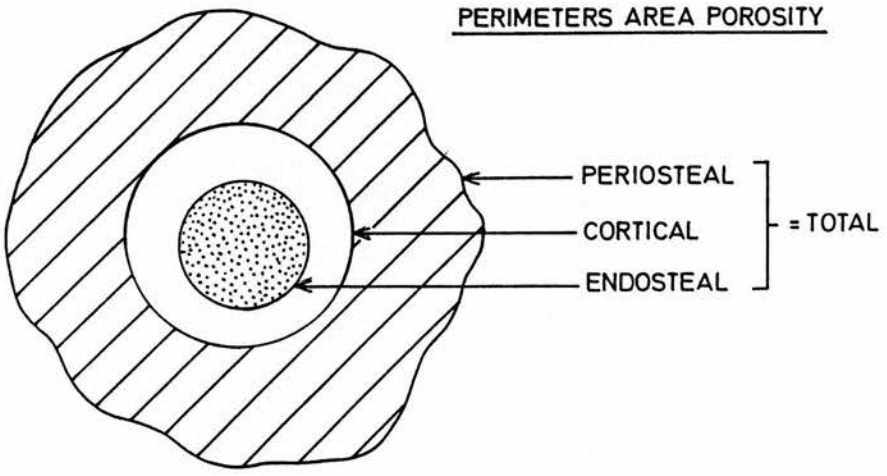
The transverse sections were analysed using Magiscan (Joyce-Loebl Ltd, Gateshead, UK), a microcomputer based image analysis system which allows either automated or interactive processing using GENIAS software.

8.4.1 Microradiographic parameters

Quantitative analysis was performed in accordance with the recommendations of the Histomorphometry Nomenclature Committee of the American Society of Bone and Mineral Research (Parfitt et al 1987). Data from primary measurements was confined to and expressed in two dimensions, thereby avoiding the assumptions required for stereological interpretation. The image analysis system was initially calibrated by placing a standard rule on the stage of the stereomicroscope, capturing an image of the millimetre scale with a video camera and deriving a scaling factor, which was recorded for each measurement.

For the calculation of endosteal, cortical, periosteal and total area the proximal and distal microradiographs of each osteotomy were viewed under low power (10x magnification) so that the complete cross-section was captured. For the purpose of this experiment, area was defined as including all tissue, mineralised and non-mineralised, enclosed within the periosteal, cortical and endosteal perimeters respectively, as illustrated in Figure 8.4. In order to correct for the inherent variability between animals in normal tibial

Figure 8.4: Diagram of regional analysis within each microradiograph



dimensions, area measurements were expressed as a fraction of the (outer) cortical perimeter which was assumed to remain constant for each animal during the experiment, and which in fact showed no significant differences between the three groups (Table 8.1). This convention is well-recognised (Parfitt et al 1987).

The standard group (group S) had a significantly greater periosteal callus perimeter than the rigid group ($p<0.05$). This finding was also supported by larger periosteal and total cross-sectional areas in Group S than in Group R, although statistical signficance was not obtained using the t-test. Compared with the standard group, in the devascularised group (Group D) there was a significant increase in endosteal perimeter ($p<0.01$) and endosteal area ($p<0.005$), together with a correspondingly lower cortical area ($p<0.005$), reflecting the prominent proximal endosteal resorption seen in the longitudinal histological sections (Table 8.1).

More detailed analysis was performed at higher magnification (40x) by dividing each microradiograph into four quadrants, from which regional porosity and relative photodensity were determined. Porosity was defined by the relationship:

$$Porosity\ (\%) = (1 - fractional\ area\ of\ mineralised\ tissue) \times 100$$

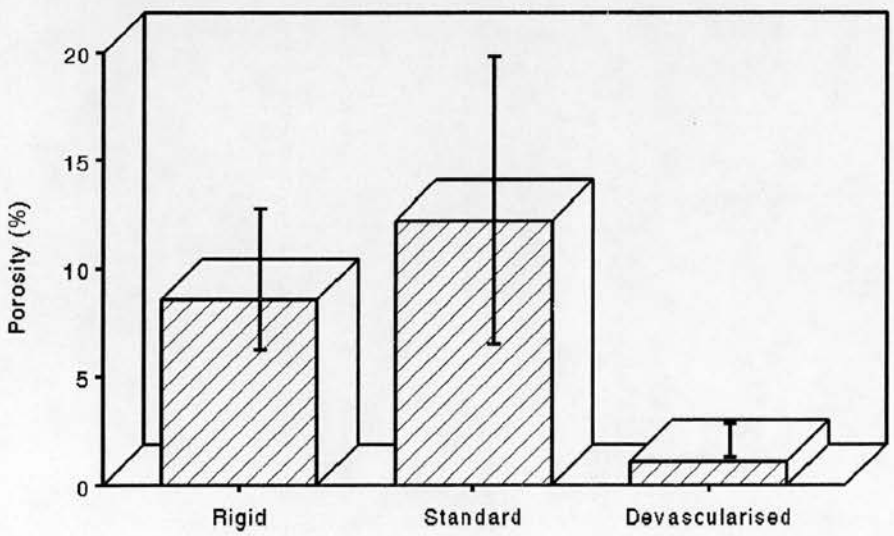
within each region, including low density mineralising osteoid. As with the regional area measurements, the detected area was determined by using an interactive routine to apply a binary image threshold to the mineralised tissue seen on the microradiograph.

There was no difference between Group S and Group R in the porosity of periosteal callus, and although the porosity of endosteal callus was statistically significantly lower in the standard group, the presence and quantity of endosteal callus available for analysis was extremely variable in these groups.

Table 8.1: Normalised area and perimeter measurements at 42 days after osteotomy

	Rigid	Standard	Devascularised
Perimeter (mm)			
Periosteal	79.02 (+/-4.56)[a]	97.28 (+/-24.11)[a]	-
Cortical	50.60 (+/-1.80)	51.35 (+/-3.25)	49.75 (+/-1.52)
Endosteal	27.77 (+/-3.12)	27.48 (+/-1.77)[b]	32.33 (+/-4.63)[b]
Normalised area (/CP)			
Total	7.85 (+/-0.99)	8.48 (+/-1.63)	-
Periosteal	4.15 (+/-1.02)	4.74 (+/-1.52)	-
Cortical	2.56 (+/-0.19)	2.66 (+/-0.22)[c]	2.06 (+/-0.39)[c]
Endosteal	1.14 (+/-0.21)	1.08 (+/-0.09)[d]	1.47 (+/-0.37)[d]

Figure 8.5: Regional cortical porosity at 42 days



Of particular note, however, was a significantly greater mean cortical porosity in Group S (12.22%) than in Group R (8.55%), (Figure 8.5). These figures compare well with those of Aro et al (1990) at a similar stage in the healing of tibial fractures in the rat. The respective area, perimeter and porosity differences are illustrated in the representative microradiographs in Figure 8.6.

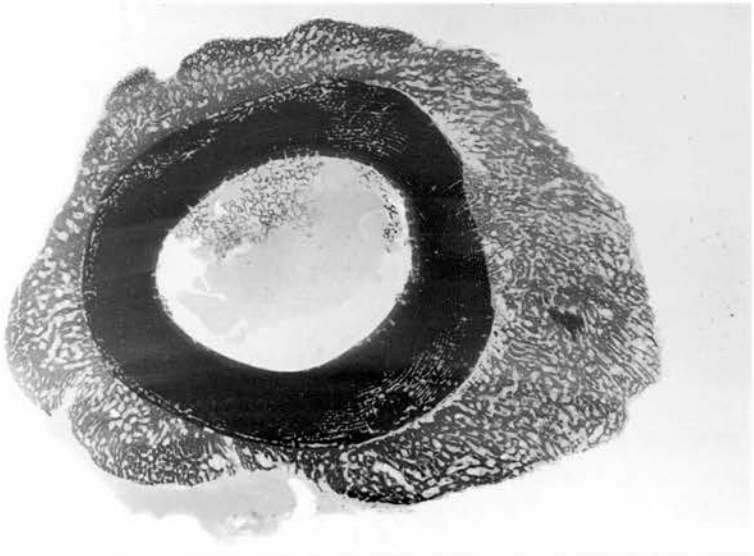
Photodensity of mineralised tissue in periosteal, cortical and endosteal bone was determined in each quadrant by random sampling of 30 single pixel energy values within each region. These were collected by the operator using an interactive light pen to select points on the high-resolution monitor. Because of the possibility of slight variation in exposure between individual microradiographs, ratios of endosteal and periosteal callus-to-cortical photodensity were compared between the well-vascularised groups. This revealed that the density of the new bone of the endosteal callus was not different from that of the periosteal callus, and at six weeks after osteotomy was equivalent to about 75% of the density of cortical bone. The photodensity of the callus of the rigid group was slightly, but significantly greater ($p<0.005$) than that of the standard group.

8.4.2 Regional mineral apposition rate

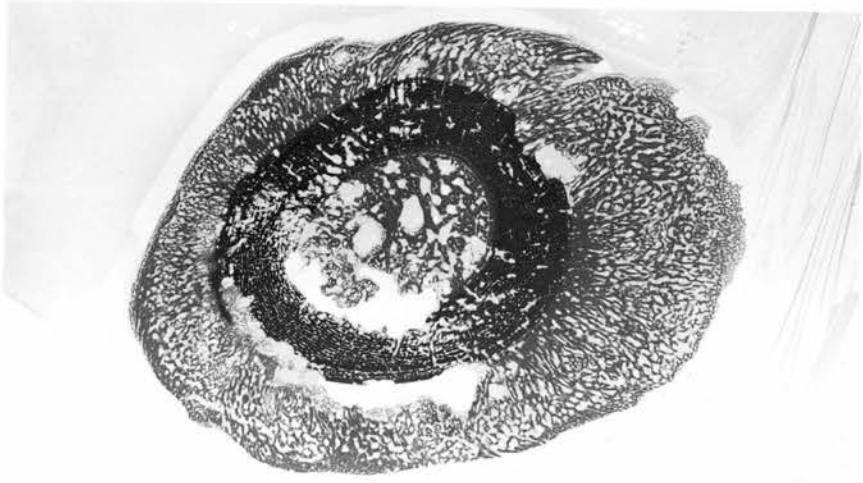
Mineral apposition rate was defined according to the nomenclature of Parfitt et al (1987), as the distance between two points on the corresponding edges of two consecutive fluorochrome labels, divided by the time between administration of the labels. The undecalcified 120 micron thick sections were viewed under a stereo microscope at low power (25x) with an incident ultraviolet light source. Initially, the endosteal, cortical and periosteal regions were examined for the presence of fluorochromes and if two or more labels were found in a parallel array, then quantitation of MAR was undertaken.

Figure 8.6 Cross-sectional microradiographs at 42 days

Rigid



Standard



Devascularised

Each section was subdivided into four quadrants for analysis at higher power (250x magnification). Using a low-light, high-resolution charge-coupled camera (Labstar, Image Processing and Vision Co Ltd, Coventry, UK) mounted on the microscope, images of each quadrant were captured by the image analysis system and displayed on a monochrome screen. For the measurement of length, a scaling factor was calibrated as described in Section 8.4.1. A series of fifteen chords was then drawn between corresponding points on consecutive edges of the labels appearing at Day 0, Day 14 and at Day 28 respectively, using an interactive routine and light pen, and the measured distance divided by fourteen days to give a MAR in microns/day. These measurements provided a mean and standard deviation from 15-60 estimations for each section. No label was given immediately prior to death, and therefore the appositional edge of new bone was used instead for calculation of the MAR from 28-42 days. There was scant evidence of any fluorochrome uptake in any of the devascularised sections (Group D) and therefore the technique was confined to the well-vascularised groups.

Qualitative examination in the early phase (0-14 days) showed that in both groups, the initial osteogenic response occurred most frequently on the endosteal surface (Figure 8.7), but in all regions labelling was found more commonly in the semirigid group (Table 8.2). By the middle of the experiment (14-28 days) periosteal mineralisation had become more prominent, being evident in nearly all the sections in both groups. In the late phase (28-42 days) there was extensive periosteal labelling accompanied by a rise in frequency of intracortical fluorescence. The prevalence of endosteal activity remained essentially the same throughout the experiment in both groups.

Calculation of the rate of mineral apposition revealed no significant differences either between groups, or between endosteal, cortical or periosteal regions, or between successive phases of the healing process within each group (Table 8.3). The MAR approximated 1-2 microns/day, and this was in agreement with the rates quoted by other

Figure 8.7: Endosteal fluorochrome labelling

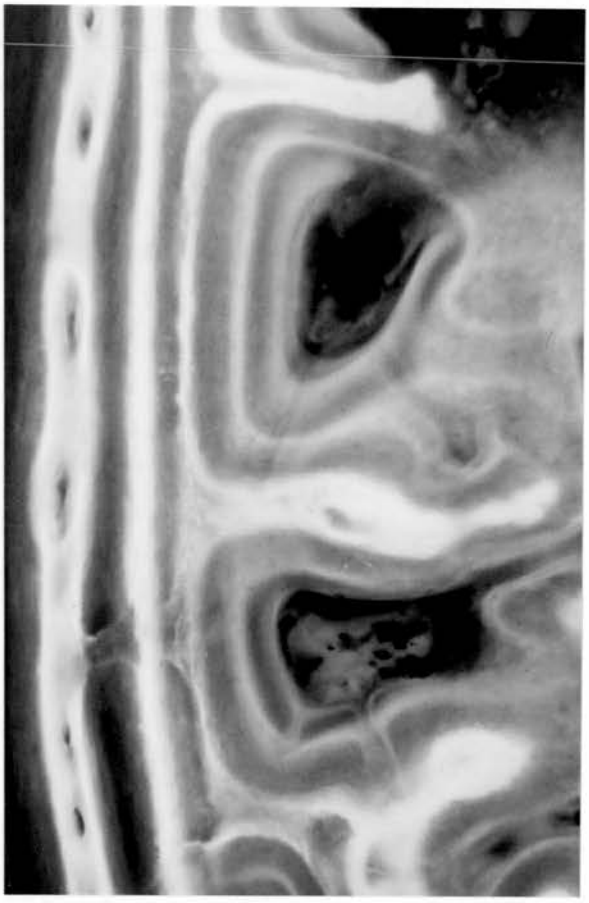


Table 8.2: Presence of fluorochrome labels (% of fields examined)

	Rigid	Standard
I: 0-14 days		
Endosteal	50	60
Cortical	12	20
Periosteal	12	30
II: 14-28 days		
Endosteal	50	70
Cortical	12	20
Periosteal	100	80
III: 28-42 days		
Endosteal	50	50
Cortical	87	80
Periosteal	100	100

Table 8.3: Regional mineral apposition rate (um/day)

	Rigid	Standard
I: 0-14 days		
Endosteal	1.56 (+/-0.66)	1.41 (+/-0.76)
Cortical	1.81 (+/-0.10)	1.27 (+/-0.08)
Periosteal	1.51 (+/-0.06)	1.59 (+/-0.17)
II: 14-28 days		
Endosteal	1.72 (+/-0.62)	2.40 (+/-0.49)
Cortical	0.94 (+/-0.06)	1.00 (+/-0.37)
Periosteal	1.82 (+/-0.27)	1.66 (+/-0.30)
III: 28-42 days		
Endosteal	1.82 (+/-0.73)	1.77 (+/-0.34)
Cortical	1.80 (+/-0.21)	1.61 (+/-0.61)
Periosteal	1.55 (+/-0.39)	1.80 (+/-0.54)

authors (Frost 1980, Einhorn et al 1990).

8.5 Summary

In the well-vascularised semirigid group (Group S), the initially higher axial gap strains and cortical blood flows were associated with similar differences in the distribution of new bone in and around the osteotomy site, in particular a greater periosteal perimeter, periosteal cross-sectional area and cortical porosity than in the rigid group (Group R). Qualitative longitudinal histological appearances were also consistent with the torsional testing results, in that there appeared to be a larger volume of callus in the semirigid group which tended to fail at the interface between the original cortex and fibrous tissue in the interfragmentary gap. The interfragmentary zone in the rigid group, by contrast, revealed a predominance of new woven bone rather than fibrous tissue, and had tended to fail partially through adjacent cortical bone, a more advanced stage in the classification of White et al (1977).

Although endosteal osteogenesis was initiated early in the healing response, this was soon superceded by synthetic activity in the rapidly developing periosteal callus (Figure 8.8). By six weeks, the original cortex had also undergone considerable resorption, either associated with or as a consequence of the early rise in cortical blood flow, resulting in increased intracortical porosity in both groups. The coupled resorptive-formative process was reflected during the late phase (28-42 days) by the increase in intracortical new bone formation as the excavated channels were filled with secondary osteons (Figure 8.9).

The mineralisation process itself, however, proceeded at a fairly uniform rate in the cortex and in the callus, which had a relative microradiographic density approximately 75% that of the cortex. These findings suggest that at the cellular level there was no fundamental difference in the material properties of newly formed bone in reponse to

Figure 8.8: Periosteal fluorochrome labelling

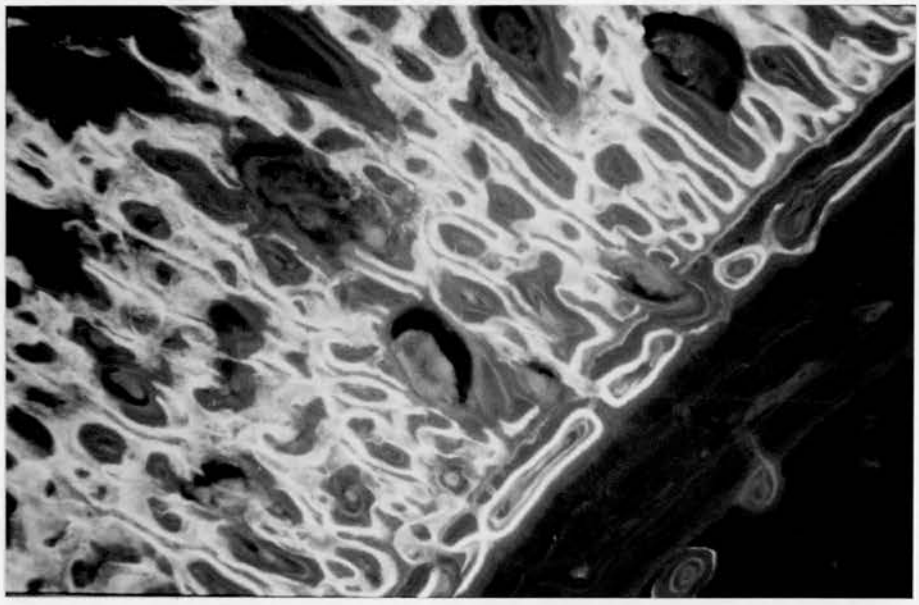
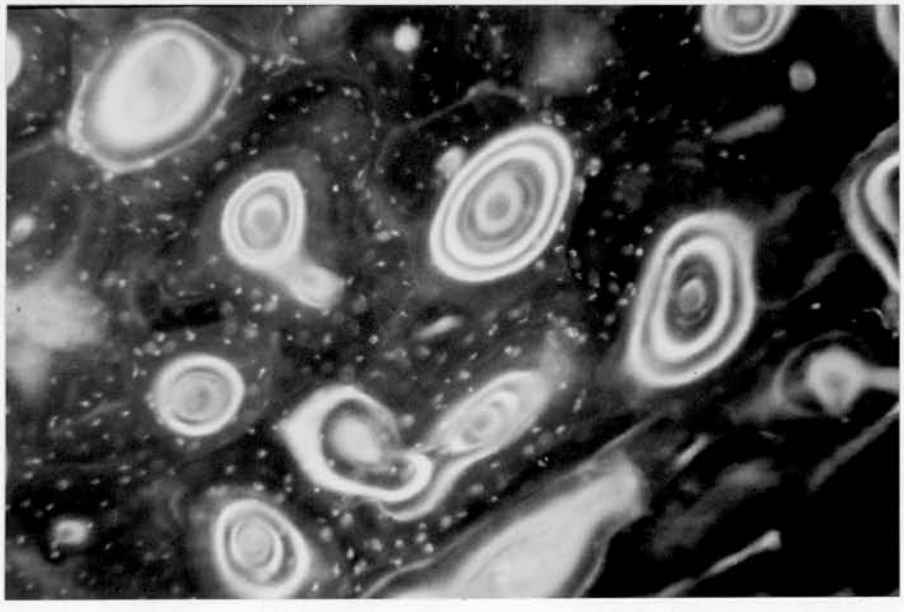


Figure 8.9: Intracortical fluorochrome labelling



different mechanical environments, but that the distribution and organisation of the repair tissue was the major factor in determining the structural properties measured by torsional testing at the six week stage, and that this was related to the pattern of the local vascular response.

Extensive cortical devascularisation (Group D) resulted in cortical necrosis and an absence of remodelling activity within the cortex, but at the endosteal surface there was marked and progressive resorption by osteoclastic cells, followed by a central core of vascularised medullary callus originating from the proximal tibia. However, this attempt at reconstruction was insufficient to create any significant mechanical linking of the proximal and distal fragments by the end of the experiment.

9. DISCUSSION

Historically, in the field of fracture research, the most extensively studied factors affecting the rate and progress of healing have been the role of mechanical loading and of afferent blood flow. Clinically, these have been recognised to be of real significance in fracture management, and yet it is surprising that so few experimental investigations have attempted to define their relative contributions and possible inter-relationship. In order to interpret the haemodynamic responses to different tissue strains, with and without a periosteal vascular reserve, the provision and monitoring of a controlled mechanical environment for this experiment was a fundamental requirement. To this end considerable effort was made to design a system which enabled progressive 'realtime' estimation of applied loads and corresponding displacement in a prescribed direction with reasonable accuracy and reproducibility.

9.1 Creation of a controlled mechanical environment

Previously, some workers have attempted to measure relative fracture gap tissue strains by inference from measured pin displacements (Kaplan et al 1985, Williams et al 1987). More recently, systems have been devised in which the applied loads and measured displacements are either a function of the combined mechanical characteristics of the fixator and the weightbearing activity of the animal (Claes 1989, Gilbert, Dahners and Atkinson 1989) or alternatively, a function of a powered actuator which loads or displaces the fragments to preset, arbitrarily determined values (Goodship and Kenwright 1985, Cheal et al 1991). In the latter type of system, physiological variables such as the pain response, which might alter gap strains by inhibition of weightbearing, are avoided. However, the possibility of interruption of local feedback mechanisms by such artificial loading regimes makes the

findings in these studies more difficult to relate to the clinical situation.

In any event, at the organ level, a fixation system which attempts to control and measure motion in any of the six degrees of freedom occurring at the fracture site necessarily results in an oversimplification of those strains which must exist at the cellular level in the callus. From experimental studies analysed with finite element modelling, Cheal et al (1991) has drawn attention to the complex strain field which developed during axial compression of interfragmentary tissue, and found shear strain gradients which occurred at the periosteal and endosteal margins where resorption was noted in vivo, as predicted by Carter (1988).

However, for practical purposes the measurement of these local strains is extremely difficult if not impossible, and the resultant bulk tissue displacement must serve as the representative quantity in any given plane. For this experiment, it was elected not to use a powered system, but to study the reduction of osteotomy axial strain in concert with recovery of limb function, as gauged from the vertical component of the ground reaction force. For simplicity, the analysis was confined to the degree of freedom of the longitudinal axis of the fixator, which was estimated to be the direction of applied loads of greatest magnitude during ambulation. Loading was sampled weekly and assumed to be representative of those loads applied by the animal during the course of healing. Interval activity was not recorded for each individual, so that the entire loading history of each osteotomy could not be quantified.

The preliminary studies in this experiment demonstrated that it was possible to measure axial loads in the bar of an external fixator applied to the osteotomised ovine tibia, that these loads decreased with time during healing, and that devascularisation of the cortex adjacent to the osteotomy resulted in an apparent change in the rate of reduction of the axial load in the bar which correlated with qualitative radiological changes at the

osteotomy site. These results provided the basis for the final design of the experimental fixator and microcomputer-based load analysis system incorporating the instrumented treadmill, which was used to calculate axial osteotomy displacement as the primary in vivo mechanical parameter in three groups of mature female sheep. The experimental design allowed for the investigation of the effects of two variables, namely (i) an increase in mean axial fixation stiffness from 240 N/mm to 460 N/mm within a well-vascularised environment, and (ii) supression of the musculoperiosteal blood supply to the osteotomised region of the tibial diaphysis, held with a mean axial fixation stiffness of 240 N/mm.

9.2 Review of experimental results

9.2.1 Osteotomy displacement and axial strain

The in vivo biomechanical measurements revealed that in the first few weeks after an osteotomy in which the soft tissues were relatively undamaged, and in which the vascular connections between the muscle, periosteum and cortex were preserved, an increase in axial fixation stiffness of 92% allowed the animals in the rigid group to achieve significantly higher weightbearing than those in the standard group. This additional loading across the osteotomy meant that the measured fixator axial loads, osteotomy displacements and gap strains were not in proportion to the difference in fixation stiffness; in fact, at fourteen days the axial displacements in the standard group were only 25% higher than in the rigid group. The other consequence of the extra loading facilitated by increased fixation stiffness was higher bone-pin interface stresses, resulting in a significantly higher rate of pin loosening in the rigid group.

In the later phase of the experiment, the curves illustrating the progressive reduction of osteotomy axial gap strain for these well-vascularised groups appeared to converge, so

that between 28 and 42 days the rate of reduction was identical, and displacements were almost indetectable by the end of the experiment. Periosteal devascularisation, however, was associated with persistently high osteotomy displacements and marked limitation of weightbearing during the late phase, indicating that the fixator was carrying significant loads even at 42 days, and that the progressive transfer of applied load to the healing bone was delayed.

9.2.2 Regional haemodynamic reponses

Although less different than predicted, the early disparity in osteotomy displacement and gap strains appeared to be associated with remarkable differences in the magnitude of the cortical and medullary vascular response in the well-vascularised groups. In this experiment, the fourfold greater blood flow at fourteen days in those osteotomies in the standard group in which 25% greater micromovement was demonstrated, suggests a high degree of sensitivity of the vessels to changes in the local mechanical environment. This differential response appears to be transient, however, paralleling the reduction in axial displacement, and by 42 days both cortical and medullary flow had returned toward normal, and was not different between the two groups. Mineral uptake, represented by the early uptake of Technetium-99m MDP and assessed by an external counting technique, appeared to increase during the experiment in both well-vascularised groups but no significant difference was found between them.

Devascularisation of the cortex in the region of the osteotomy by interposition of a silicone rubber sleeve between bone and muscle was shown to result in dramatic attenuation of the cortical vascular response, even in the presence of similar axial displacements. While this model represented an extreme insult when compared to the clinical situation, it avoided the introduction of confounding variables such as the difficulty of quantifying a

reproducible muscle injury or the effect of release of intracellular components from tissue necrosis. In the early phase, medullary blood flow appeared to be relatively unaffected by suppression of the periosteal reserve, and continued to increase throughout the experiment. This was not accompanied by changes in mineral uptake, which remained significantly lower than in both well-vascularised groups.

9.2.3 Mechanical properties in torsion

While the measured haemodynamic responses were impressive, in principle the goal of fracture healing is the restoration of the structural integrity of the bone in order to allow a return to function, and therefore one of the most important outcome measures in this experiment was the mechanical testing in torsion. In the well-vascularised groups, the finding that there were no significant differences in any parameter at 42 days, demonstrated that within such an optimal vascular environment, the effect of the disparate fixation stiffnesses was to produce, via different pathways or mechanisms, a similar structural 'endpoint' in this experiment (although full recovery of mechanical properties had not occurred). On the other hand, interruption of the periosteal blood supply severely curtailed functional recovery, to the extent that at 42 days the mechanical properties in the devascularised group were approximately equivalent to those measured at fourteen days in the well-vascularised groups.

9.2.4 Distribution of new bone

The marked differences in haemodynamic responses appeared to provide strong evidence for a role for the vasculature in these mechanisms of strain reduction which resulted in the common endpoint in the well-vascularised groups. Qualitative and quantitative analysis of

the distribution of new bone formation was therefore carried out in the form of standard radiography, routine longitudinal histology, cross-sectional microradiography and fluorescent histomorphometry. Ordinary lateral radiographs showed relatively more periosteal callus in the standard group, associated with a persistent radiolucent line at the ostotomy site at 42 days, than in the rigid group in which this was less frequent. The devascularised group showed no periosteal new bone, but some evidence of medullary callus, which was mainly in the proximal fragment.

Histological observation of a representative longitudinal section from each group at 42 days supported these findings, and revealed that in the standard group, which had recorded greater blood flow and micromovement in the early phase, failure had occurred through the largely fibrous gap tissue (White Stage II) and the callus. In the rigid group failure had occurred partially through woven bone in the gap and partially through the original cortex (White Stage III). In the devascularised group, endosteal cortical bone had been actively resorbed in the proximal fragment and a conical core of medullary callus had formed within a fibrous envelope, but had not reached the level of the osteotomy by 42 days and therefore contributed little to mechanical stability in torsion.

Cross-sectional analysis yielded quantitative data which were in agreement with these qualitative findings. It was evident that callus area and perimeter, and cortical porosity (12%) were slightly but significantly greater in the standard group than in the rigid group. This evidence, when considered in the light of the large early blood flow differences between the two groups, suggests the ingrowth of new intracortical vessels at the sites of bone resorption, in addition to increased flow in pre-existing vessels or recruitment of normally non-perfused 'resting' capillaries within the cortex (Hughes 1978) which might also contribute to the increased flow measured after osteotomy.

In relation to the degree of micromovement and mechanical testing, the histological and

microradiographic data reflected the distribution of new bone and other tissues. Higher early gap strains (65%) appeared to result in prominent rises in early blood flow, followed by formation of a relatively large volume of callus, considerable cortical resorption and development of interfragmentary fibrous tissue. Lower early gap strains (40%) appeared to favour a more modest flow response, less callus, less cortical resorption and more rapid differentiation of interfragmentary woven bone. At the final period chosen for comparative testing, however, these morphological differences were secondary to the fact that in structural terms, the two groups of well-vascularised osteotomies had achieved essentially equal status.

The effect of periosteal devascularisation and imposition of a physical barrier to prevent revascularisation from extra-osseous sources effectively eliminated cortical flow adjacent to the osteotomy gap, and there was evidence of widespread cortical necrosis. The huge rise in medullary blood flow appeared to precede the induction of extensive resorption, which was demonstrated in the cross-sections from the osteotomy by significantly increased endosteal and decreased cortical area and perimeter. The resorption appeared to be confined to the endosteal surface only, because cortical porosity remained similar to normal, intact bone (<2%). Medullary callus was not seen in the cross-sections, because the front of new bone in the proximal fragment had not reached the level of the osteotomy.

9.3 The role of fixation and micromovement

The findings in this experimental study provide further information concerning several fundamental issues in the discussion of fracture healing. One of the most significant from a clinical point of view is the importance of fixation rigidity and its dependent variable, the relative micromotion or strain occurring at the fracture site. Gilbert, Dahners and Atkinson (1989) argued that in the well-vascularised experimental osteotomy,

the effect of fixation rigidity was irrelevant in fracture healing until fragment movement was so excessive as to result in nonunion. In a retrospective study of open tibial fractures, Court-Brown et al (1990) suggested that clinical outcome was independent of the biomechanical characteristics of the device chosen for fixation. However, Kenwright et al (1991) have shown in a prospective trial that daily applied micromovements of up to one millimetre resulted in a more rapid return to independent weightbearing in non-comminuted open tibial fractures treated with external fixation.

The evidence from the well-vascularised groups in this study suggests that differences in the imposed mechanical environment provided by an external fixator may be attenuated early in the repair phase by the external loading consequent upon the weightbearing activity of the animal. The greatest axial strain recorded in this experiment was about 65%, and this seems to represent the physiological upper limit of fragment displacement, which may be mediated by a feedback mechanism involving pain sensation in the interfragmentary or periosteal tissue. The concept of feedback regulation has also been postulated in relation to weightbearing after tibial fracture in man (Kenwright et al 1991).

While it proved possible to produce significantly different strain fields using the instrumented device in this study, the resultant effect appeared to be early and transient, as suggested by Williams et al (1987). Within three weeks the rate of strain reduction was almost identical in the well-vascularised groups, and by six weeks there was essentially no difference in terms of structural or functional recovery, although histologically and microradiographically the distribution of repair tissues was different. Hence, under such ideal biological conditions, the callus was able to cope with a reasonably wide range of initial mechanical environments by influencing the load applied to the fractured bone and by varying the nature of the differentiating repair tissue.

The histological findings are in general agreement with the interfragmentary strain theory

of Perren (1979), in that gap strain was progressively reduced and there appeared to be a greater amount of new interfragmentary bone in the group with less micromotion. Claes, Wilke and Rubenacker (1989) attempted a similar experiment in the ovine metatarsal using an external fixator and claimed to produce strains ranging from 0-75%, but concluded that Perren's concept could not be validated from their experiment. Cheal et al (1991) also found no consistent relationship between the stresses predicted in the interfragmentary tissue from finite element modelling and the amount of local cortical resorption. The complexity of the strain field occurring in a transverse osteotomy subject to axial loading means that with current technology, attempts to correlate measurements made at the macroscopic level with events occurring during tissue differentiation at the microscopic level result in oversimplification.

From a clinical point of view, the value of in vivo monitoring using instrumented devices is also of relevance in discussion of the role of micromovement in fracture healing. In a well-vascularised environment, the transience of the effect of fixation rigidity would appear to limit the usefulness of such techniques, as also found by Kaplan et al (1985) who used a tensile loading system. However, in the devascularised osteotomy, the failure of strain reduction was clearly demonstrated as early as four weeks after injury. In clinical practice, early detection of delay in the loadbearing ability of devascularised fractures by instrumented devices may allow earlier surgical intervention using such methods as bone grafting to augment the biological response.

9.4 The role of the vascular response

In the present experiment, the effect of micromovement, when considered strictly in terms of structural and functional recovery, appeared to be limited. Investigation of the early vascular response in cortical bone, however, demonstrated a high degree of sensitivity to

the micromovement created by the initial mechanical environment. Smith et al (1990) compared cortical blood flow in canine tibial osteotomies held with external fixators, intramedullary nails and compression plates and found differences at fourteen days, with the greatest flows recorded in the externally-fixed group; however the problem with such experiments is that the vascular effect of the application and presence of the devices themselves cannot be separated from the effect of the different mechanical environments they produce.

It could not be directly ascertained from the present study whether the elevated cortical and medullary blood flows recorded were specifically a consequence of higher flow in vessels normally perfused at baseline levels, or de novo angiogenesis, or both. Nonetheless, the early differences in flow were succeeded by changes in cortical porosity and periosteal cross-sectional area which suggested a close relationship between the vascular response and the organisation of new bone formation.

Angiogenesis has recently been well-characterised using both in vivo and in vitro models, in the search for new anti-angiogenic therapies for preventing tumour growth in cancer (Presta and Rifkin 1988), and for control of proliferative vascular conditions such as those occurring in diabetes mellitus and rheumatoid arthritis (Zetter 1988). Unfortunately, the role of angiogenic factors has not been well described in healing bone, although the growth of new vessels in and around the osteotomy site occurs early in the repair process, probably initiated within seven days (Hasan and Brookes 1990).

Three phases of vessel growth have been described which seem to apply in both physiological and pathological states in diverse tissues. The first phase is the disruption of the basement membrane of the capillary, which may be influenced by the release of enzymes such as collagenase from the endothelial cells themselves. The second phase is migration of the exposed endothelial cells toward the angiogenic stimulus,

possibly on a fibrin scaffold, followed in the third phase by proliferation of the succeeding cells to form a new capillary bud and re-establishment of the basement membrane (Zetter 1988, Presta and Rifkin 1988).

This sequence may be affected by both mechanical and chemical factors, and these may be acting either synergistically or competitively in the early fracture callus. Blood pressure, blood flow, capillary wall tension and shear stress, and distortion by surrounding tissues have all been implicated in stimulating the growth of new vessels (Hudlicka and Tyler 1986). In wound healing, induction of angiogenesis also appears to be mediated by inflammatory macrophages (Fruhbeis et al 1988). These cells may be activated by hypoxia (Taylor et al 1988), the presence of toxins (Hunt et al 1984), and by growth factors such as interleukin-1 and tumour necrosis factor-alpha (Takahashi et al 1991). In models of endochondral ossification, the events of angiogenesis, chondrolysis and calcification occur simultaneously at around nine days (Reddi et al 1989), and have been associated with localisation of basic fibroblast growth factor-beta at the mineralisation front (Joyce et al 1991).

The haemodynamic changes demonstrated in the present experiment may simply reflect a *passive* increase in supply in response to the metabolic demands of differentiating osteogenic cells, or may reflect an *active* role for the vasculature in the proliferative or synthetic processes, determining the distribution of new bone. Blood flow has previously been correlated with remodelling activity in intact bone (Sim and Kelly 1970) and with new bone formation in cortical defects (McInnis, Robb and Kelly 1980), which led the authors to conclude that the bone capillary played a purely nutritional role.

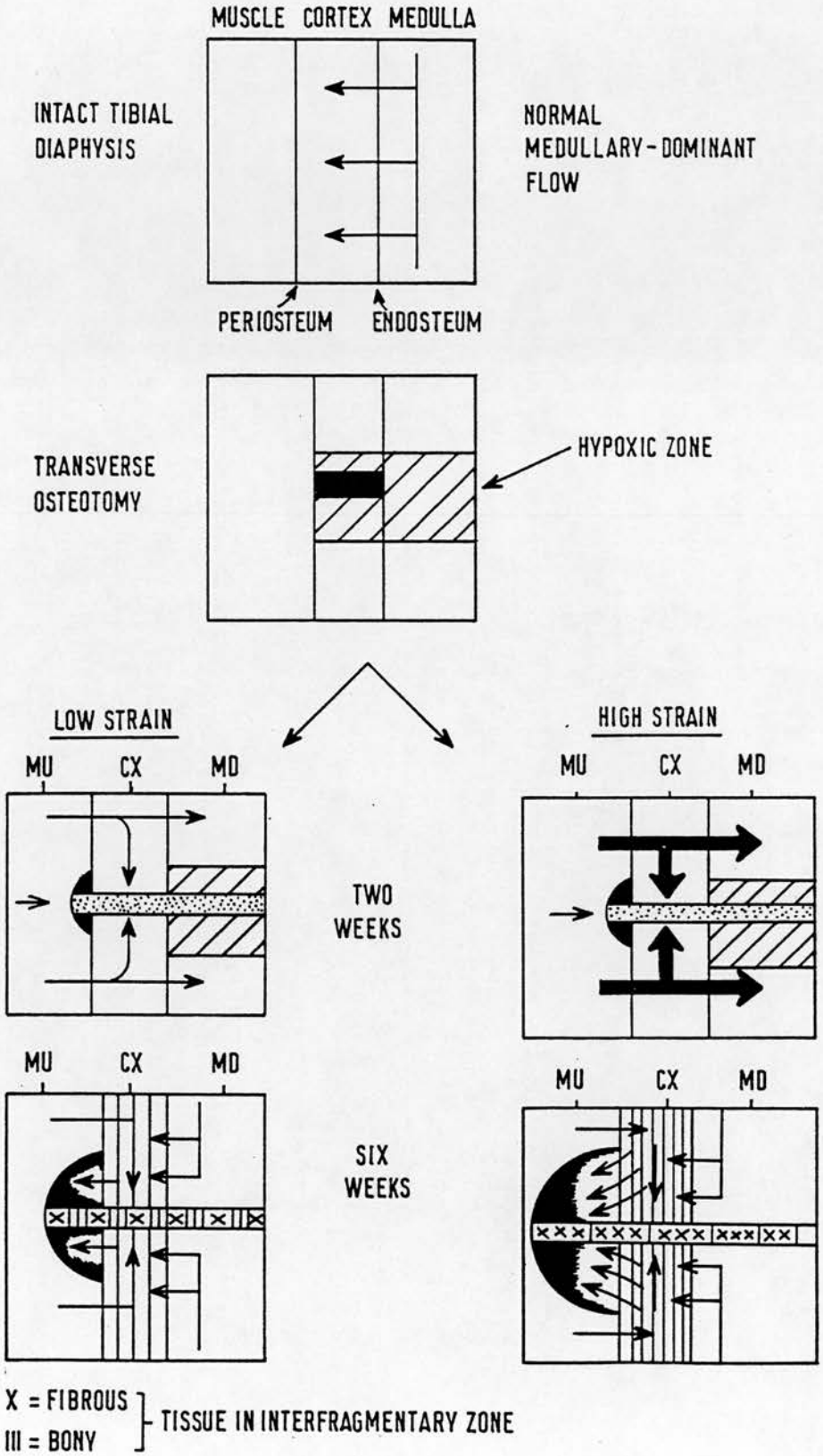
Although the mechanism is not clear, there is direct and indirect evidence in the literature to support the concept of angiogenically-mediated osteogenesis. The idea that blood vessels may form bone was first proposed by John Hunter (1794) from his observations

on the growth of deer antlers. In the early 1960s, Trueta argued that the bone capillary formed a syncytium with the mature osteocytes and was the source of osteogenic cells. Chidgey et al (1986) found a correlation between the qualitative and quantitative vascular changes occurring after osteotomy of the canine radius and the return of rigidity in four-point bending. Shapiro (1988) described the dependence of the repair of cortical defects in the rabbit upon undifferentiated mesenchymal cells accompanying blood vessels, the ingrowth of which began to diminish at three weeks.

More recently, Brighton and co-workers (1991) have demonstrated a constant spatial relationship of blood vessels with enlarged endothelial cells, polymorphic mesenchymal cells, osteoblasts and foci of new bone in the medullary fracture callus of the rabbit. In discussing these findings, the authors go so far as to suggest that transformed endothelial cells extravasate from the capillary to become the pericytes, or polymorphic mesenchymal cells. Indeed Streeten and Brandi (1990) have recently argued that as in other tissues, endothelium in bone may possess distinctive, organ-specific functions.

A new hypothesis for the role of the vascular response is presented in Figure 9.1, in which the osteotomy site is represented by a three-compartment model. Following injury, there is division of the medullary arteries within the diaphysis, resulting in hypoxia of the medulla and cortex adjacent to the interfragmentary gap, particularly in the distal fragment. This environment, augmented by the arrival of inflammatory cells in the haematoma, acts as a potent stimulus for induction of angiogenesis from surrounding muscle capillary beds, and a vascular granulation tissue migrates into the gap region. Interfragmentary axial movement results in shearing of the newly forming matrix, and to distortion and repetitive microdamage to the budding capillaries at the fragment margins. This produces a massive release of vasodilator metabolites and angiogenic factors from both the matrix and the damaged endothelial cells, which is initially related to the magnitude of the interfragmentary strain, but which acting by positive feedback or

Figure 9.1: The effect of mechanical strain on regional vascular responses after fracture



autoregulation, for example by enhanced sensitivity of the endothelium to these local stimuli, facilitates an accelerated response. The consequence of this reaction is to cause, within fourteen days, (i) increased flow in existing intracortical channels, augmented by periosteal-cortical anastomoses, and (ii) proliferation of periosteal osteoblasts, and (iii) a stimulus for new vessel growth within the cortex and callus.

As a result of these changes, between two and six weeks the periosteal callus rapidly enlarges and there is a drive to local remodelling of the cortex. Existing cortical vascular channels are initially widened by osteoclastic resorption, and new vascular channels appear, causing an increase in intracortical porosity. The intracortical vascular network is now the major supply to the callus and interfragmentary zone and receives contributions from both the periosteal and medullary systems, the latter of which is becoming re-established. The reduction of interfragmentary movement is progressively achieved by a mineralisation front at the interface of the periosteal callus and the interfragmentary tissue. The size of the callus is related to the intracortical porosity, but as the quantity of woven bone in the interfragmentary zone increases and movement is reduced, intracortical and medullary blood flow declines, and the enlarged channels begin to fill with lamellae of new bone, forming new osteons.

This hypothesis serves to explain the haemodynamic and histomorphometric changes observed in the present study, and is consistent with the qualitative investigations of Teneff (1950), who demonstrated that the regenerating blood vessels supplying the callus arose not only from the adjacent soft tissues, but from the cortical fragment ends as well. An increase in intracortical porosity at six weeks after a tibial fracture was also noted by Aro et al (1990b), in association with the development of radial channels entering the cortex from the medullary cavity. Although the authors could not explain this cortical porosis, it was thought unlikely to be a consequence of stress-protective osteopenia.

While it is interesting to speculate about the underlying mechanisms responsible for the osteogenesis in the well-vascularised experimental osteotomies used in the present study, the other important aspect of the investigation was the specific role of the afferent periosteal vascular reserve. Although the model used represented an extensive and severely devascularised cortical insult, healing by medullary callus was slow and mechanically inferior, despite an impressive vascular response within the marrow itself and the measurement of osteotomy displacements comparable with those in the well-vascularised environment. Revascularisation of the osteotomy site was originating from the proximal fragment, as noted also by Richards (1991) in a devascularised canine model, presumably because of the continued viability of the nutrient arterial system. In the osteotomised rabbit tibia held with an external fixator which maintained a gap, Brueton et al (1990) found ischaemia of the distal fragment which was only revascularised after medullary vascular union had occurred at six weeks after osteotomy.

Rapid restoration of bony integrity, irrespective of the mechanical conditions of the fracture, therefore appears to be heavily dependent on the viability of the periosteal blood flow, which was quantitatively shown to be the predominant source of supply to the osteotomy site in the early phase of healing by Strachan et al (1990). Holden (1972) demonstrated that revascularisation of muscle preceded cortical revascularisation in devascularised fractures, although the mechanical environment in his study was not quantified or monitored. Bensusan (1990) has stated that the prevailing vascularity of a bone graft has a more significant effect on the progress of healing than the effect of a change in stiffness of the fixation plate with which it is stabilised.

These experimental studies underline clinical observations that damage to the extrinsic musculoperiosteal supply, as occurs during sustained elevation of intracompartmental pressure, delays fracture healing (Court-Brown and McQueen 1987). Surgical intervention to restore this reserve, such as by early muscle flap coverage (within 7-14 days) of

severe open tibial fractures, seems to be associated with more rapid radiological union, lower infection rates, and decreased hospitalisation than with delayed coverage (Fischer et al 1991).

9.5 Conclusions

The healing of diaphyseal fractures is a complex process involving the differentiation of a number of tissues in response to mechanical and biological factors acting at the fracture site. The coordinated formation of callus appears to operate via local feedback mechanisms, resulting in revascularisation, new bone formation and reduction of interfragmentary movement in preparation for definitive remodelling of the injured bone to its original state. The single most important factor determining the progress of the healing reponse is suspected to be the rate of recovery of the periosteal vascular reserve, augmented by its anastomoses with adjacent muscle capillary beds after injury. If this reserve is suppressed over a large extent of cortex, healing by medullary callus is slow and union is delayed.

In a well-vascularised environment, angiogenesis appears to precede and be intimately related to the osteogenic process, providing evidence to support the endothelial cell and its derivatives as osteogenic precursors (Trueta 1963). The magnitude of angiogenesis, as inferred from quantitative measurements of blood flow, seems to be strongly influenced by the magnitude of the axial gap strain. The early formation of new bone, determined by the vascular response, then results in progressive reduction of osteotomy displacement; these steps in the cascade of repair would appear to be initiated within two to three weeks after the original injury.

The sensitivity of the vascular response to mechanical stimuli, particularly in the early

phase of healing, means that studies in which biological variables are being investigated must provide a carefully controlled mechanical environment, the contribution of which should be monitored progressively throughout the experiment. If the truly dynamic nature of the interplay between mechanical and biological factors is not appreciated, serious errors of interpretation might occur, particularly in the critical early phase when intercellular feedback mechanisms are thought to be active.

9.6 Recommendations for further work

The logical extension of these studies can be considered either in terms of further investigation of mechanical factors affecting the fracture site, or alternatively in terms of further characterisation of biological variables implicated in the osteogenic process. In planning any further experiments, both must be taken into consideration.

The evidence from this study and those of previous investigators suggests that there is no narrowly-defined optimal mechanical environment for fracture healing. Indeed the coordinated differentiation of pluripotential mesenchymal cells seems to be able to manage a wide range of axial strains, at least up to 65%. The lower end of the range, however, continues to present a paradox: Perren's interfragmentary strain theory predicts that the lower the gap strain, the more rapidly new bone should be able to differentiate; however, clinical and experimental evidence also shows that high degrees of fracture site rigidity (implying very low strains) are associated with suppression of the healing response. There is still a great deal of work, therefore, which could be done to provide a definitive understanding of the role of micromovement, particularly in relation to its application to different fracture configurations, but it must be remembered that the status of the periosteal vasculature seems to be much more significant in determining the progression of healing, and therefore the potential application of such work to clinical

practice will be limited by the extent of bone and soft tissue devascularisation associated with each fracture.

The devascularised insult used in this experiment represented the 'worst-case scenario' which would not be observed frequently in clinical practice. An interesting and more directly clinically applicable approach would be to combine a controlled mechanical environment, perhaps using several different strain fields, with reproducible and quantifiable muscle injury, in order to model more closely the human situation.

Further investigation of the angiogenic-osteogenic association promises to be very rewarding, with potential application to other aspects of orthopaedic surgery such as osseous reconstruction and biocompatibility, and porous ingrowth or coated joint prostheses. At the organ level, the identification and synthesis of specific angiogenic factors and their therapeutic use in augmenting revascularisation of bone and muscle may prove to be more appropriate to the healing of fractures than the current focus on growth factors involved in the control of cortical remodelling. At the cellular level, measurement of the secretion of angiogenic factors in response to local changes in the mechanical environment may yield valuable information.

The early phase of fracture healing is increasingly being recognised as the key stage in determining outcome, both in terms of osseous restitution and in terms of function for the patient. This means that there is a 'therapeutic window' of probably only one to two weeks after a fracture in which the surgeon has the opportunity to restore the biological environment, so that the influence of the mechanical conditions will be maximal. Therapeutic intervention to accelerate diaphyseal fracture healing remains a realistic objective, as long as this fundamental inter-relationship is appreciated. It would appear that better understanding of the relationship of mechanical and biological factors in this critical early period could result in a considerable saving of healthcare resources, fewer

secondary operative procedures and a more rapid rehabilitation of the injured patient.

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11. APPENDICES

11.1 Tabulated data

The following tables refer to the graphs depicted in the main text, and contain the relevant mean and standard deviations for each parameter for each group. Significant differences are referred to in the appropriate section of the main text.

Figure 5.2: Ground reaction force

Postoperative Day	Rigid	Standard	Devascularised
(i) Absolute values (N)			
7	168 (+/-38)	134 (+/-63)	118 (+/-80)
14	127 (+/-47)	128 (+/-42)	128 (+/-85)
21	133 (+/-33)	124 (+/-24)	65 (+/-38)
28	115 (+/-24)	133 (+/-28)	63 (+/-44)
35	103 (+/-43)	147 (+/-23)	83 (+/-48)
42	139 (+/-32)	165 (+/-22)	71 (+/-32)
(ii) Normalised (% body weight in N)			
7	33.2 (+/-4.7)	24.8 (+/-10.8)	21.4 (+/-11.6)
14	25.6 (+/-6.9)	23.8 (+/-7.3)	23.5 (+/-13.3)
21	28.6 (+/-4.2)	23.6 (+/-3.9)	14.9 (+/-8.5)
28	25.7 (+/-7.8)	25.4 (+/-5.0)	14.4 (+/-9.9)
35	23.4 (+/-10.9)	28.4 (+/-6.9)	18.9 (+/-10.9)
42	31.0 (+/-8.2)	31.8 (+/-5.9)	16.5 (+/-7.8)

Figures 6.6 and 6.7: Osteotomy site regional blood flow

	Rigid	Standard	Devascularised
(i) 14 days			
Cortical	5.35 (+/-2.15)	19.54 (+/-10.59)	1.70 (+/-1.65)
Medullary	14.62 (+/-7.38)	51.74 (+/-14.22)	31.20 (+/-21.47)
Muscle	2.16 (+/-0.40)	3.56 (+/-2.45)	3.06 (+/-2.10)
(ii) 42 days			
Cortical	4.62 (+/-2.13)	2.47 (+/-0.36)	3.75 (+/-3.78)
Medullary	16.14 (+/-8.45)	26.22 (+/-16.31)	75.25 (+/-48.72)
Muscle	2.15 (+/-1.13)	2.64 (+/-0.82)	2.30 (+/-1.36)

Figure 6.10: Effect of fixator +/- osteotomy on Tc-99m MDP uptake

	----------Corrected count rate----------		
Minutes after injection	Normal	Fixator only	Standard
1	0.064 (+/-0.009)	0.087 (+/-0.018)	0.194 (+/-0.065)
5	0.059 (+/-0.004)	0.089 (+/-0.008)	0.302 (+/-0.118)
10	0.059 (+/-0.005)	0.091 (+/-0.009)	0.365 (+/-0.148)
15	0.061 (+/-0.003)	0.093 (+/-0.011)	0.410 (+/-0.171)
20	0.060 (+/-0.005)	0.092 (+/-0.012)	0.437 (+/-0.185)
25	0.060 (+/-0.004)	0.093 (+/-0.014)	0.464 (+/-0.198)
30	0.060 (+/-0.004)	0.093 (+/-0.013)	0.474 (+/-0.200)
AUC 0-1 minute	0.581 (+/-0.424)	0.521 (+/-0.081)	1.048 (+/-0.275)
AUC 5-15 minutes	0.662 (+/-0.052)	1.006 (+/-0.100)	3.869 (+/-1.505)

Figures 6.11 and 6.12: Osteotomy site Tc-99m MDP uptake

	Corrected count rate		
Minutes after injection	Rigid	Standard	Devascularised
(i) 14 days			
1	0.124 (+/-0.034)	0.194 (+/-0.065)	0.137 (+/-0.049)
5	0.186 (+/-0.047)	0.302 (+/-0.118)	0.174 (+/-0.053)
10	0.226 (+/-0.058)	0.365 (+/-0.148)	0.193 (+/-0.060)
15	0.253 (+/-0.063)	0.410 (+/-0.171)	0.206 (+/-0.069)
20	0.270 (+/-0.069)	0.437 (+/-0.185)	0.208 (+/-0.074)
25	0.283 (+/-0.072)	0.464 (+/-0.198)	0.211 (+/-0.079)
30	0.294 (+/-0.075)	0.474 (+/-0.200)	0.211 (+/-0.082)
AUC 0-1 minute	0.675 (+/-0.233)	1.048 (+/-0.275)	0.723 (+/-0.286)
AUC 5-15 minutes	2.460 (+/-0.620)	3.869 (+/-1.505)	2.141 (+/-0.743)
(ii) 42 days			
1	0.136 (+/-0.021)	0.161 (+/-0.048)	0.149 (+/-0.043)
5	0.247 (+/-0.029)	0.280 (+/-0.093)	0.185 (+/-0.043)
10	0.319 (+/-0.045)	0.353 (+/-0.123)	0.205 (+/-0.045)
15	0.368 (+/-0.059)	0.408 (+/-0.150)	0.212 (+/-0.046)
20	0.403 (+/-0.067)	0.449 (+/-0.166)	0.221 (+/-0.049)
25	0.432 (+/-0.079)	0.487 (+/-0.178)	0.224 (+/-0.050)
30	0.457 (+/-0.088)	0.517 (+/-0.188)	0.231 (+/-0.056)
AUC 0-1 minute	0.682 (+/-0.141)	0.810 (+/-0.312)	0.793 (+/-0.256)
AUC 5-15 minutes	3.465 (+/-0.486)	3.800 (+/-1.411)	2.234 (+/-0.497)

Figures 7.4 and 7.5: Torsional properties

Property	Rigid	Standard	Devascularised
(i) Maximum torque (Nm)			
Osteotomy - 14 days	1.17 (+/-0.41)	1.41 (+/-0.37)	NR
Osteotomy - 42 days	12.72 (+/-4.08)	21.20 (+/-15.18)	0.35 (+/-0.47)
Control tibia - 14 days	68.88 (+/-7.61)	73.53 (+/-15.97)	60.77 (+/-4.84)
Control tibia - 42 days	60.70 (+/-9.77)	66.41 (+/-11.97)	59.54 (+/-3.10)
(ii) Torsional stiffness (Nm/degree)			
Osteotomy - 14 days	0.06 (+/-0.03)	0.07 (+/-0.02)	NR
Osteotomy - 42 days	0.91 (+/-0.16)	1.05 (+/00.28)	0.01 (+/-0.02)
Control tibia - 14 days	2.00 (+/-0.68)	2.62 (+/-0.58)	1.85 (+/-0.18)
Control tibia - 42 days	1.98 (+/-0.33)	2.14 (+/-0.24)	1.98 (+/-0.10)
(iii) Energy absorbed to failure (J)			
Osteotomy - 14 days	0.52 (+/-0.29)	0.45 (+/-0.25)	NR
Osteotomy - 42 days	2.10 (+/-1.34)	5.23 (+/-6.14)	0.05 (+/-0.06)
Control tibia - 14 days	26.81 (+/-8.90)	23.03 (+/-7.86)	20.69 (+/-3.39)
Control tibia - 42 days	22.31 (+/-6.09)	21.59 (+/-5.81)	18.73 (+/-2.00)
(iv) Angular deformation (degrees)			
Osteotomy - 14 days	42.86 (+/-13.10)	35.68 (+/-8.92)	NR
Osteotomy - 42 days	17.58 (+/-6.37)	22.42 (+/-13.68)	46.91 (+/-23.74)
Control tibia - 14 days	45.73 (+/-18.22)	39.26 (+/-3.88)	41.07 (+/-6.70)
Control tibia - 42 days	41.19 (+/-6.83)	37.93 (+/-3.17)	37.63 (+/-1.18)

NR = not recordable

Figure 8.5: Regional cross-sectional porosity

	Rigid	Standard	Devascularised
Periosteal	44.08 (+/-8.12)	41.64 (+/-8.99)	-
Cortical	8.55 (+/-3.25)	12.22 (+/-6.65)	1.12 (+/-0.77)
Endosteal	56.49 (+/-12.99)	43.33 (+/-12.62)	-

11.2 Published work

THE EFFECT OF DEVASCULARISATION UPON EARLY BONE HEALING IN DYNAMIC EXTERNAL FIXATION

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We examined the effect of periosteal devascularisation upon the early healing of osteotomies of sheep tibiae held in an instrumented external fixation system with an axial stiffness of 240 N/mm. At 14 days, cortical blood flow measured by the microsphere technique was 19.3 ml/min/100g in the well-vascularised osteotomies, but only 1.7 ml/min/100g in the devascularised osteotomies, despite an increase in medullary flow ($p < 0.0005$). Delay in healing of the devascularised osteotomies was suggested by an in vivo monitoring system and confirmed by post-mortem mechanical testing. We suggest that the osteogenic stimulus of dynamic external fixation is dependent on the early restoration of cortical blood flow in devascularised fractures.

It has long been recognised that the healing of diaphyseal fractures is related to the severity of damage to the bone and soft tissues at the time of injury (Ellis 1958; Oestern and Tscherne 1984). The fracture configuration, the presence of infection, the blood supply of the fragments and the stability of fixation all have an effect upon the rate of fracture union (Court-Brown and Hughes 1982; Gustilo, Mendoza and Williams 1984).

The term 'biomechanical environment' has been used to describe the influence of fixation devices upon the histological pathways of fracture union (Chao et al 1989), but in a wider sense it may include the effect of biological variables implicit in the healing process, such as changes in oxygen tension (Heppenstall, Goodwin and Brighton 1976), blood flow (McCarthy and Hughes 1984), neural mechanisms (Aro, Eerola and Aho 1985), electrical potential (Law et al 1985), and osteo-inductive factors in the extracellular matrix (Reddi, Wientroub and Muthukumaran 1987).

Investigators have attempted to define an 'optimal' biomechanical environment in terms of the direction and magnitude of loading which will stimulate osteogenesis. In the isolated intact avian ulna, Rubin and Lanyon (1987) showed that bone mass may be maintained by as little as four cycles of loading per day, and a maximal osteogenic response was produced by 36 cycles per day, though this was dependent on the strain distribution within the bone.

Yamagishi and Yoshimura (1955) showed, in an externally-fixed osteotomy of the rabbit tibia, that moderate intermittent axial compression resulted in more rapid healing than did shear forces. Wolf et al (1981) used similar experiments to discover a threshold of cyclic axial loading, below which no demonstrable effect on healing occurred.

The introduction of the first clinical external fixator to allow axial loading of healing callus (De Bastiani, Aldegheri and Brivio 1984) has been followed by experimental and clinical attempts to control the amount of loading and consequent fragment displacement (Goodship and Kenwright 1985; Kenwright and Goodship 1989). These have generated the concept of an optimal 'window' of axial fixation stiffness (Perren 1979).

Much of the experimental evidence for the beneficial effect of axial micromovement has relied on well-vascularised osteotomy models, whereas the clinical problems of delayed and nonunion are frequently associated with severe open fractures (grades II, III), in which there is extensive periosteal stripping (Court-Brown et al 1990).

The importance of the osteogenic stimulus of dynamic external fixation in fractures with a poor vascular supply is not known. Studies which have endeavoured to alter the afferent blood flow to healing fracture models, are not comparable because of differing

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systems of fixation, affecting the mechanical environment (Olerud and Danckwardt-Lilliestrom 1971; Whiteside and Lesker 1978; Richards and Schemitsch 1989) and altering the blood flow to cortical bone at the fracture site (Smith, Bronk and Kelly 1990).

The objectives of this study were to examine quantitatively the effect of periosteal devascularisation on the healing of the osteotomised sheep tibia, held in an instrumented external fixation system which allowed measurement of axial fixator stiffness over a period of two weeks. This early stage was chosen as the time at which blood flow is at its peak (Paradis and Kelly 1975) and the capillary surface area is maximal from hypertrophy and regeneration (Hughes et al 1978). Bone mineral deposition, determined by uptake of the tracer technetium-99m methylene diphosphonate, has been found to increase significantly two weeks after osteotomy in the tibiae of dogs (McCarthy and Hughes 1984). At this stage blood flow to the healing callus appears to be largely independent of the endosteal supply (Strachan et al 1990), although the timing of re-establishment of normal centrifugal circulation, as conceived by Brookes et al (1961), has never been accurately defined.

MATERIAL AND METHODS

A pilot study was undertaken to design an experimental fixation system. A unilateral fixator was used, with stiffness allowing an axial load of 1000 N, to produce a combined deflection of less than 1 mm. The fixator bar was composed of one solid piece with integral strain-gauge transducers to measure the axial load and the bending moment in the plane of the pins.

From these measurements a modular fixator was devised (Fig. 1). It consists of a main body with two sets of three bone pins, connected by linear bearings (RSR-15M, THK Co, Tokyo, Japan) which allow movement only in the axis of the fixator. The pin-holding blocks are separated by a spring-transducer module which determines passive axial micromovement. The spring is constructed of room-temperature-vulcanising silicone (Cosmesil Silicone, Cosmedica, Cardiff, Wales) and an epoxy resin spacer; by varying the dimensional ratio of these components, a wide range of linear stiffness can be accurately prescribed. A transducer measures the axial load on the fixator bar; since the spring stiffness is known, the axial displacement at the osteotomy can be calculated.

Displacement at the osteotomy site due to pin bending was reduced by increasing the diameter of standard 110 mm self-tapping pins (Orthofix, Verona, Italy) from 6 to 10 mm, using a tapered stainless steel sheath. Pin loosening was controlled by regular tightening with a calibrated torque wrench (Torqueleader, MHH Engineering, Bramley, England) to greater than 2 N or 2.5 N for metaphyseal and diaphyseal bone respectively.

Direct measurement of loads on the osteotomised bone is very difficult if not impossible. It is assumed that

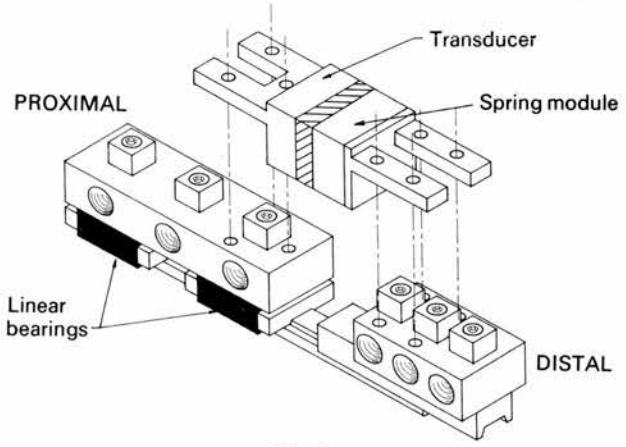

Fig. 1

Schematic diagram of modular axial fixation system.

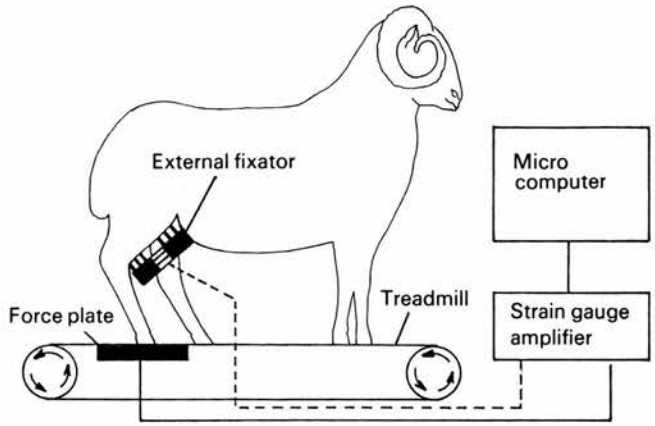

Fig. 2

Schematic diagram of in vivo monitoring apparatus.

the axial load on the tibia, due to the mass of the animal and to muscle activity, remains roughly proportional to the ground reaction force during normal walking. A measure of healing is given by the fracture stiffness index (FSI) calculated in the following way:

$$\text{Fracture stiffness index} = \frac{\text{ground reaction force}}{\text{fixator axial load}}$$

As healing progresses, and the bone carries proportionately more load, the FSI should increase. The vertical component of the ground reaction force was measured using a force-plate mounted under the belt of a treadmill (Fig. 2). At a velocity of approximately 0.5 m/sec, readings were taken from between 50 and 100 consistent steps. Signals from both the fixator bar and the force-plate were simultaneously recorded at a frequency of 50 Hz using a microcomputer (BBC Model B, Acorn Computers, Cambridge, England).

Experimental procedure. Fourteen female blackface sheep, averaging three years of age and weighing 45 to

65 kg, walked on the instrumented treadmill, after several training sessions. Thereafter they were randomly allocated to one of two equal groups: standard (well-vascularised) or devascularised. Each sheep was then intubated under general anaesthesia (halothane/nitrous oxide), given 1.5 g intravenous cefuroxime, and the fixator applied to the anterior aspect of the tibia using a special jig. The distance of the bar from the bone was 60 mm. The mean (± standard deviation) axial stiffness of fixation chosen for this study was 240 ± 5 N/mm, with stiffnesses in all other planes high enough to ensure that other deflections were insignificant. Pins were applied through stab incisions after pre-drilling using a 4.8 mm bit and saline irrigation.

In the seven sheep in the standard (well-vascularised) group, a longitudinal incision was made over the medial subcutaneous border of the right tibia. The soft tissues were carefully protected with minimal extra-periosteal disturbance. The periosteum was incised transversely at a level 65% of the length of the tibia distal from the stifle joint. The external fixator having been applied as above, a transverse osteotomy was performed between the innermost pins with a Gigli saw, leaving a 2 mm gap.

A similar osteotomy was performed in the other seven sheep but in these it was followed by circumferential stripping and excision of the periosteum for 20 mm proximally and distally. A 40 mm sleeve of silicone (internal diameter 12.5 mm, wall thickness 1.25 mm; Mackay and Lynn, Edinburgh, Scotland), was placed over both fragments to prevent revascularisation of the underlying cortex from the surrounding soft tissues. Wound closure was performed in layers using an absorbable suture of 3/0 Dexon.

The animals were given cefuroxime 500 mg by intramuscular injection, for three days after operation. Weight-bearing was allowed from the day of operation and the osteotomy was monitored two, seven and 14 days postoperatively. Each animal was then anaesthetised and lateral radiographs of the leg taken. Through a midline incision in the neck, the right carotid artery was exposed and cannulated with a Hilal-65 aortic catheter, passed into the left ventricle using a pressure transducer for reference. A measured activity of Cobalt-57 labelled microspheres (diameter 15 ± 0.5μm, half-life 267 days; DuPont Inc, Stevenage, England) was injected into the left ventricle over 30 seconds. Sufficient was administered to achieve approximately 100 to 150 microspheres per sample, which according to the work of Li, Bronk and Kelly (1989) allows estimation with an error of less than 10%. A reference sample was collected from a second catheter in the right brachial artery, attached to a withdrawal pump (Harvard Apparatus, Edenbridge, England), at a fixed rate of 0.852 ml/min for two minutes.

Each sheep was killed with 10 ml saturated potassium chloride, delivered into the ventricle. The hindlimbs were disarticulated through the stifle joints and all the soft tissues were carefully removed. Samples of muscle, and metatarsal cortical bone and marrow were taken from each side for blood flow estimation.

Mechanical testing. The ends of the osteotomised and the intact contralateral tibiae were mounted in cups using Wood's metal and each preparation was placed in a torsional testing machine. The fixators were removed from the osteotomised tibiae only after the bones were secured, to avoid accidental damage to the early callus. In the devascularised group, the silicone sleeve was incised longitudinally and removed without disturbing the medullary contents. As each pin was removed, a swab sample was taken from the pin track and sent for bacteriological culture. All bones were tested to failure within two hours of death. Measurements of applied torque and angular displacement were made, to permit calculation of maximum torque (torsional strength), torsional stiffness and energy absorbed to failure.

Measurement of regional blood flows. After mechanical testing, the metaphyses, with their cancellous bone and ligamentous attachments, were discarded to avoid overestimation of cortical bone blood flow. The diaphysis was then stripped of periosteum and sectioned transversely at 1 cm intervals. Superficial periosteum and fibrous tissue were removed from the surface of the early callus. The sections were divided into cortical and medullary fractions which, together with muscle from both compartments at the level of the osteotomy and metatarsal samples were each counted for 300 seconds in a gamma scintillation counter (LKB-Wallac, Turku Oy, Finland). Blood flows were determined from the ratio of tissue to reference sample activity, which may be arranged to give the formula:

$$\text{Flow (ml/min/100 g)} = \frac{\text{tissue activity} \times \text{withdrawal rate} \times 100}{\text{reference activity} \times \text{tissue mass}}$$

Statistical significance within each group, between osteotomised and intact tibiae, was assessed using the paired Student's *t*-test. Comparison between groups was made using the unpaired *t*-test.

RESULTS

All sheep were weight-bearing on the first day after operation. Complications were few. Clinical pin-track infection occurred in three of the 84 pins while 48 yielded positive cultures of predominantly mixed Gram-negative organisms, in spite of antibiotic prophylaxis. The remaining 33 pins were sterile. One sheep in the standard group sustained a fracture through the lowermost pin hole on the first postoperative day, and was therefore excluded from further consideration.

Although there was no statistically significant difference between the groups during in vivo monitoring, the trend indicated delay in healing in the devascularised group. In both groups the ground reaction force and stance-phase duration (time the hoof was on the ground) were lowest on the second postoperative day and

Table I. In vivo monitoring data expressed as mean(± standard error) in vascularised and devascularised tibia at 14 days

	Ground reaction force (Nm)		Stance phase duration (s)		Osteotomy displacement (mm)	
	Standard	**Devascularised**	**Standard**	**Devascularised**	**Standard**	**Devascularised**
Pre-operative	234 (± 12)	243 (± 23)	0.62 (± 0.05)	0.66 (± 0.05)	–	–
Day 2	95 (± 16)	102 (± 19)	0.38 (± 0.05)	0.44 (± 0.03)	0.99 (± 0.12)	0.96 (± 0.10)
Day 7	147 (± 29)	164 (± 24)	0.47 (± 0.05)	0.48 (± 0.05)	1.08 (± 0.17)	1.44 (± 0.14)
Day 14	152 (± 24)	193 (± 20)	0.52 (± 0.04)	0.55 (± 0.04)	1.25 (± 0.21)	1.57 (± 0.14)

increased progressively to more than 50% of the pre-operative level by the 14th day (Table I). As weight-bearing increased in both groups, the devascularised group demonstrated a greater displacement of the osteotomy (mean 1.57 mm ± 0.14). There was also a slower increase in the FSI in the devascularised group (Fig. 3).

There was no difference between the mean blood flow of medulla and cortex of the tibial diaphyses of the two groups, although there were statistically significant increases in flow when compared to the contralateral intact tibiae (Table II). Medullary diaphyseal flow demonstrated a great increase to over 20 ml/min/100g, and cortical diaphyseal flow increased by a factor of five to more than 8 ml/min/100g in both groups of osteotomised tibiae. At the osteotomy site, medullary flow again demonstrated significant increases in both groups compared to an equivalent level in the non-osteotomised bones. Cortical flow at the osteotomy site, however, was significantly lower in the devascularised group ($p < 0.0005$), remaining at 1.70 ml/min/100g (± 0.58) compared to 19.30 ml/min/100g (± 3.38) in the standard group (Fig. 4).

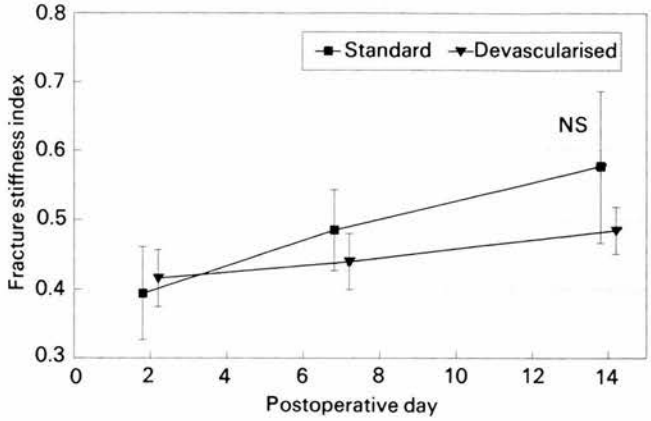

Fig. 3

Fracture stiffness index at 14 days, expressed as mean (± standard error).

Table II. Regional blood flows expressed as mean (± standard error) ml/min/100 g at 14 days

	Tibial diaphysis		Metatarsal diaphysis		Muscle compartments	
	Cortex	**Marrow**	**Cortex**	**Marrow**	**Anterior**	**Posterior**
Standard						
Experimental	8.22 ± 1.40	24.92 ± 4.15	0.60 ± 0.25	1.31 ± 0.43	3.35 ± 0.67	4.01 ± 0.79
Control	0.75 ± 0.14	0.65 ± 0.12	0.32 ± 0.09	0.87 ± 0.21	1.93 ± 0.19	2.27 ± 0.25
Significance (p)	< 0.0005	< 0.0005	NS	NS	< 0.05	< 0.05
Devascularised						
Experimental	8.40 ± 1.85	21.23 ± 3.04	0.53 ± 0.16	1.03 ± 0.21	2.98 ± 0.72	3.14 ± 0.75
Control	1.46 ± 0.65	0.78 ± 0.20	0.47 ± 0.21	0.60 ± 0.23	2.70 ± 1.03	2.57 ± 0.71
Significance (p)	< 0.0005	< 0.0005	NS	NS	NS	NS

Muscle blood flow was similar in both compartments in both limbs in the devascularised group. In the standard group, there appeared to be a 'steal phenomenon' in favour of the experimental limb, with significantly increased flow ($p < 0.05$) in both compartments in which there was no barrier to cortical revascularisation from the periosteum and adjacent muscle (Table II). Metatarsal flow showed no differences, suggesting that the increases in medullary and cortical flow were confined to the bone with the osteotomy.

Mechanical properties. Following removal of soft tissues, there was evidence of external callus enveloping the fragments in the standard group. After removal of the silicone sleeve in the devascularised group, there was little more than organising haematoma to be found lying in the osteotomy gap and medullary canal. Up to 90°

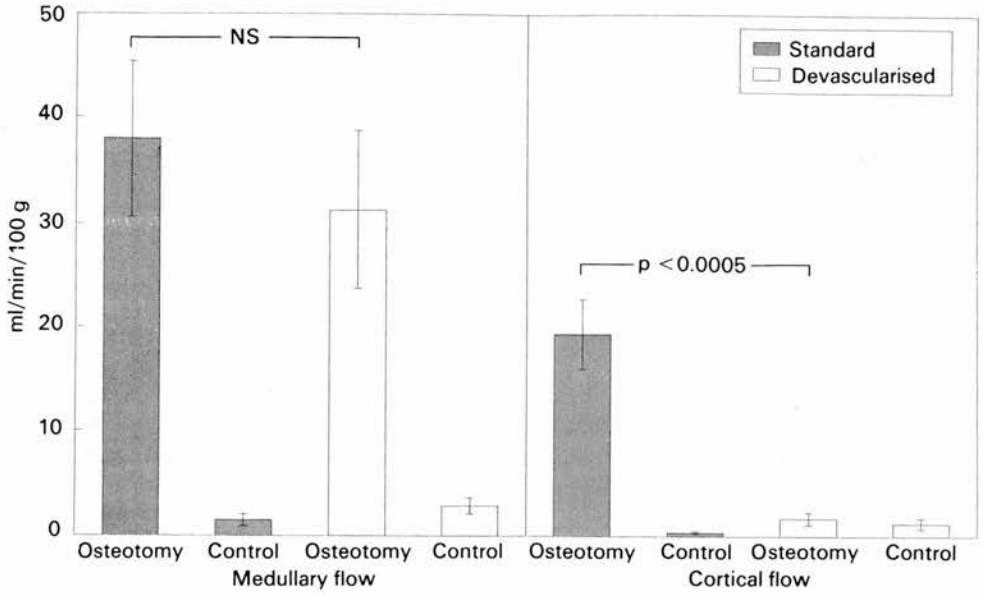

Fig. 4

Blood flow at the osteotomy site at 14 days and equivalent level in intact contralateral control bones, expressed as mean (± standard error).

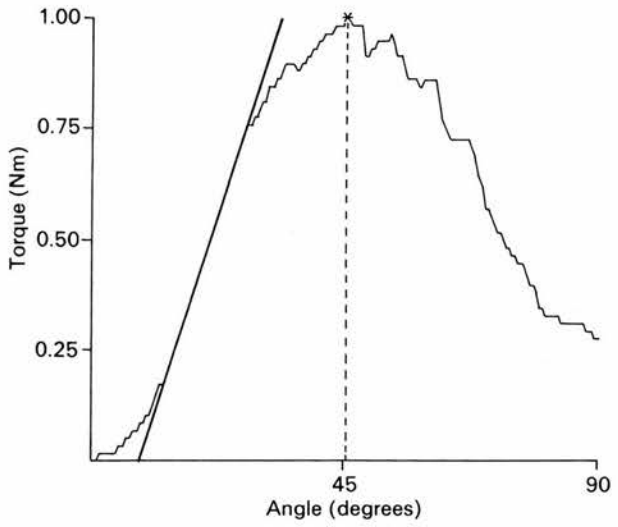

Fig. 5

Torsional properties of a standard (well-vascularised) osteotomy at 14 days (* = maximum torque).

Table III. Mechanical properties of standard (well-vascularised) group expressed as mean (± standard deviation) at 14 days

	Control	Osteotomy	Osteotomy as % of control
Maximum torque (Nm)	71.36 ± 13.19	1.17 ± 0.47	*1.63*
Torsional stiffness (Nm/degree)	2.55 ± 0.51	0.06 ± 0.02	*2.28*
Energy to failure (Joules)	23.41 ± 7.86	0.35 ± 0.25	*1.52*
Angular displacement (Degree to failure)	38.99 ± 3.48	33.58 ± 7.67	*86.62*

rotation, there was no measurable torsional resistance in any of the seven devascularised osteotomies (< 0.02 Nm). The well-vascularised osteotomies, by contrast, exhibited a consistent linear slope to the torque-angular deformation curve prior to 'failure' (Fig. 5) with a mean 1.63% of the torsional strength of the intact contralateral bones (Table III).

The angular displacement to failure of 86.62% of the non-osteotomised bones suggests the presence of 'rigid' tissue. The energy absorbed to failure of 1.52% reflects the proportionately low volume of this material. However, it appears to fail at an angle close to that of non-osteotomised bone, and therefore probably represents areas of mineral deposition or new-bone formation, even at a very early stage of healing.

DISCUSSION

Following an experimental osteotomy, the normal vascular response of bone is an increase in flow which peaks at approximately two weeks. Flow is increased not only at the site of the osteotomy, but throughout the cortex and marrow of the diaphysis (Rhinelander 1968). Our results in the standard osteotomies confirm this phenomenon, as did previous measurements in our laboratory using the canine model (Strachan et al 1990). The increased perfusion would seem to be more than adequate for delivery of nutrients to the healing tissue.

However, human tibial fractures are usually associated with some degree of damage to the soft tissues surrounding the bone, even in closed fractures (Oestern and Tscherne 1984). Muscle has been shown to provide an important collateral source of blood to cortical bone

in both clinical (Byrd, Cierny and Tebbetts 1981) and experimental studies (Richards and Schemitsch 1989). Interference with perfusion of muscle may result in delayed union in tibial fractures (Court-Brown and McQueen 1987). Whiteside and Lesker (1978) demonstrated mechanical evidence of delayed union in rabbit osteotomies associated with muscle trauma.

When the medullary vessels have been divided by osteotomy or a displaced fracture, the periosteal circulation, augmented by vascular anastomoses from adjacent muscle, assumes dominance and results in centripetally-directed flow through the cortex (Rhinelander et al 1968; MacNab and de Haas 1974). This mechanism is clearly at risk in the event of significant muscle injury. The devascularised model used in our experiments demonstrates the vulnerability of cortical blood flow when the periosteum is circumferentially destroyed, and angiogenesis from muscle is prevented. Although such an extreme vascular insult is unusual in clinical practice, this study demonstrates the importance of an extra-osseous blood supply in the early stages of healing.

Medullary flow, although increased at the osteotomy site, did not appear to provide more than basal levels of cortical perfusion in the devascularised model used in this study. Mechanical testing revealed no evidence of medullary callus whereas, in the standard group, there was 'rigid' tissue in a well-vascularised external callus. In experiments on callus distraction in the rabbit, Kojimoto et al (1988) gave histological evidence of medullary callus formation at ten days after osteotomy, but we were not able to confirm this in the sheep model.

In vivo monitoring provided encouraging results at this early stage of healing and gave clearer evidence than radiographs of the delay in healing in the devascularised group. This was confirmed by mechanical testing. Further experiments at a later stage of healing are required but recent clinical studies have demonstrated the usefulness of similar techniques (Cunningham et al 1988). In vivo monitoring depends not only on the integrity of connections within the fixator assembly, but perhaps more importantly on a firm link between the pins and the bone, where plastic deformation due to pin bending may occur under axial loads (Klip and Bosma 1978). Because resistance to bending is a function of the fourth power of the pin radius, increasing the pin width significantly increases its stiffness (Kempson and Campbell 1981) and the validity of in vivo monitoring.

Our experiments suggest that in the early stage of fracture healing, enhancement by axial loading of the callus may depend fundamentally on the musculoperiosteal vascular reserve. The fixation system selected for this study relies on passive transmission of axial loads through the osteotomy site which will vary slightly from step-to-step and between different animals in the same group. Other methods of achieving dynamic external fixation include 'elastic' fixation which is reliant upon the pins bending under load, and active controlled displacements using mechanical actuators (Aro and Chao 1990). The calculated initial displacements in this study are consistent with those found to stimulate osteogenesis in the sheep tibia by Goodship and Kenwright (1985).

It has been suggested that with rigid external fixation, the clinical outcome of severe open tibial fractures is independent of the device chosen (Court-Brown et al 1990), but reflects the degree of devascularisation of the fractured bone. Clearly further attempts to quantify the vascular and mechanical parameters are required. Our experiments indicate that in early healing, the establishment of an optimal 'biological' environment, to revascularise cortical bone, for example, by muscle coverage, is necessary to maximise the osteogenic potential of dynamic external fixation.

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INTRACAPSULAR FRACTURES OF THE NECK OF FEMUR

PARALLEL OR CROSSED GARDEN SCREWS?

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The results of treatment in 242 patients with intracapsular fractures of the neck of femur treated with Garden screws are presented with reference as to whether the screws were crossed or parallel.

The incidence both of nonunion and of avascular necrosis was less in those fractures treated with parallel screws. The outcome was also superior if the reduction was good.

A variety of implants are available for the fixation of intracapsular hip fractures. Crossed Garden screws (Fig. 1) have been extensively used (Garden 1964; Barnes et al 1976) but there is now a tendency to use parallel screws or pins, to allow for collapse at the fracture site (Strömqvist et al 1983; Linde et al 1986). We have compared the use of crossed Garden screws with a subsequent series using parallel Garden screws (Fig. 2).

PATIENTS AND METHODS

We reviewed the notes and radiographs of all patients in whom an intracapsular fracture of the hip had been fixed with Garden screws from January 1979 to December 1985. Patients who had died soon after operation or had been followed up for less than two years were excluded, leaving 242 patients for the study. All operations had been performed using image intensification and a fracture table. When indicated, the fractures were reduced by closed manipulation and then fixed, generally by a percutaneous technique.

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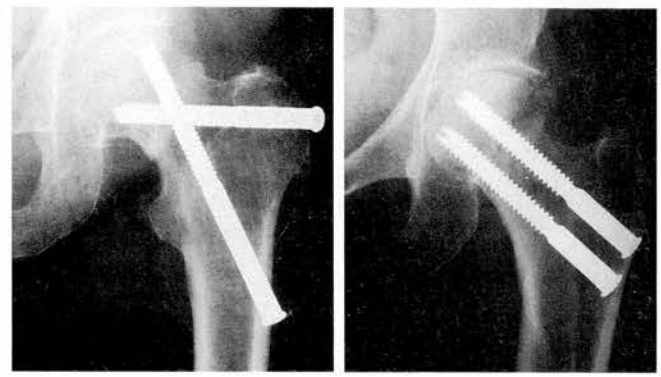

Fig. 1 Fig. 2

The placement of the screws: Figure 1 – crossed, Figure 2 – parallel.

The fractures were graded according to Garden (1961). Reduction was assessed by measuring the angles between the trabeculae on both anteroposterior and lateral radiographs. If residual angulation was less than 10° on both projections then reduction was classed as good; otherwise it was defined as poor. The position of the screws, crossed or parallel, was recorded and also the incidence of avascular necrosis, nonunion, and the length of follow-up.

RESULTS

Garden screws were placed in parallel in 88 patients and crossed in 154. The characteristics of the two groups of patients are shown in Table I. Reduction was classed as good in 87 cases (36%) but there was a difference between the two groups; 47 patients (31%) of the crossed group and 40 (45%) of the parallel group had a 'good' reduction.

The incidence of nonunion and avascular necrosis is shown in Table II. The rate of nonunion was 15% in the